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У черговому випуску журналу представлені оригінальні роботи з технології лікарських препаратів, статті з синтезу, реакційної здатності та аналізу біологічно активних речовин та лікарської рослинної сировини. Розглянуті актуальні питання організації та економіки фармації, висвітлені деякі аспекти експериментальної фармакології.

Для науковців, провізорів, лікарів, організаторів системи охорони здоров'я.

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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 ETHYL 3-ALKYL-2-(ALKYLTHIO)-5-METHYL-4-OXO-3,4-DIHYDROTHIENO[2,3-*d*]PYRIMIDINE-6-CARBOXYLATE DERIVATIVES

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Key words: thiophene; pyrimidine; mercaptans; alkylation; cyclization

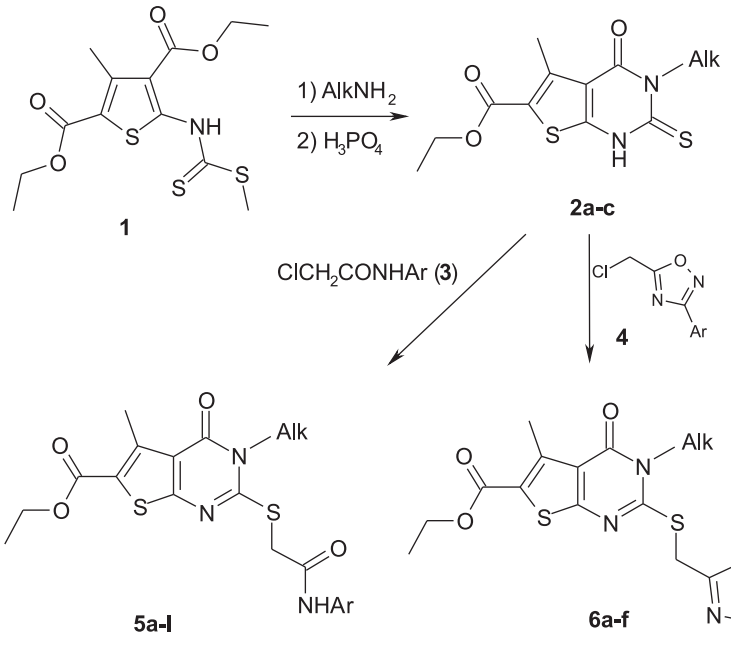
The effective method for the synthesis of ethyl 3-alkyl-5-methyl-4-oxo-2-thioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-6-carboxylate derivatives by interaction of diethyl 3-methyl-5-{[(methylsulfanyl) carbothioyl]amino}thiophene-2,4-dicarboxylate with low aliphatic amines in the 2-propanol medium has been developed. The conditions proposed facilitate isolation and perceptibly improve the yields of the target thiones. The further modification of ethyl 3-alkyl-5-methyl-4-oxo-2-thioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-6-carboxylate has been performed by alkylation with chloroacetamides and 3-aryl-5-(chloromethyl)-1,2,4-oxadiazoles (DMF-triethylamine). The structure of the compounds obtained has been confirmed using the NMR spectroscopic methods; the products of alkylation have the signals of the carbethoxy group as two signals in the ranges of 1.27-1.30 ppm (3H, t) and 4.24-4.29 (2H, q), and the signal of SCH_2 protons in the range of 4.22-4.93 ppm. The study of the antimicrobial activity for the functionalized derivatives of thieno[2,3-d]pyrimidine, the corresponding ethyl 3-alkyl-5-methyl-2-({2-[arylamino]-2-oxoethyl}thio)-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxylates and ethyl 3-alkyl-5-methyl-4-oxo-2-{[(3-aryl-1,2,4-oxadiazol-5-yl)methyl]thio}-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxylate has shown their moderate antimicrobial properties, while for some compounds with the n-butyl substituent at position 3 possess the high inhibitory activity against Candida albicans fungi growth.

Derivatives of 2-thiothieno[2,3-*d*]pyrimidine-6-carboxylic acid are known as the various biologically active compounds [6, 7, 10], antimicrobials are also found in this range of compounds [12, 13]; therefore, development of their preparation methods is an up-to-date problem of modern organic synthesis. The particular attention is also paid to the molecules containing small substituents, their presence favourably increases their drug-likeness [9]. A convenient approach towards the synthesis of 2-thiothieno[2,3-*d*]pyrimidines is application of xanthogenates as intermediates [2, 3, 6, 11, 12], but according to the conditions proposed, in the cases of using primary amines the reaction requires boiling in dimethylformamide (DMF) with further dilution with water for crystallization of the product. Unfortunately, for the low aliphatic amines, which have lower boiling points than the solvent and also may contain a huge amount of water, such reaction conditions may not be suitable. Though the effectiveness and homogenicity of this reaction, dilution with water does not help to isolate the desired products, it may be caused by the high water solubility of the target thione salts with aliphatic amines. Therefore, the aim of this work was to improve the conditions for the synthesis of ethyl 3-alkyl-5-methyl-4-oxo-2-thioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-6-carboxylates for their further modification via the alkylation reaction.

Materials and Methods

Chemical Part

All of the solvents and reagents were obtained from the commercial sources. Meting points (˚C) were determined with a Kofler (Hotbench) melting point apparatus. ^{1}H NMR spectra were recorded with a Bruker Avance drx 500 (500 MHz) spectrometer in DMSO-d_6, using TMS as a standard. Chemical shifts (δ) are reported in ppm. LC/MS spectra were recorded using a chromatography/mass spectrometric system consisting of a high-performance liquid chromatograph equipped with a diode-matrix and mass-selective detector. The method of chemical ionization under atmospheric pressure (APCI) was used. Ionization mode with simultaneous scanning of positive ions was in the mass range of 80-



Scheme

1000 m/z. Elemental analysis was performed by Kjeldahl method.

The starting **diethyl 3-methyl-5-{[(methylsulfanyl) carbothioyl]amino}thiophene-2,4-dicarboxylate** (**1**) was obtained using the previously reported methods [1, 8, 11].

The general method for the synthesis of compounds 2. To the suspension of **1** 5 g (0.014 Mole) in 2-propanol (30 ml) add 0.021 Mole of the corresponding amine. Reflux the reaction mixture for 3 h and after cooling dilute with water. Neutralize the solution obtained with H_3PO_4, filter the precipitate formed and wash with plenty of water and 2-propanol.

Ethyl 3,5-dimethyl-4-oxo-2-thioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-6-carboxylate (2a). M.p. – 266-267°C. Yield – 73%. ^{1}H NMR (*DMSO-d_6*) δ: 1.27 (3H, t, $OCH_2C\underline{H}_3$); 2.65 (3H, s, CH_3); 3.52 (3H, s, NCH_3); 4.23 (2H, q, $OC\underline{H}_2CH_3$).

LC/MS: m/z (MH^+) 285.2. Found, %: N 9.97. $C_{11}H_{12}N_2O_3S_2$. Calculated, %: N 9.85. M.w. 284.36.

Ethyl 3-ethyl-5-methyl-4-oxo-2-thioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-6-carboxylate (2b). M.p. – 253-254°C. Yield – 89%. ^{1}H NMR (*DMSO-d_6*) δ: 1.17 (3H, t, $NCH_2C\underline{H}_3$); 1.27 (3H, t, $OCH_2C\underline{H}_3$); 2.69 (3H, s, CH_3); 4.24 (2H, q, $OC\underline{H}_2CH_3$); 4.33 (2H, q, $NC\underline{H}_2CH_3$); 13.71 (1H, s, NH).

LC/MS: m/z (MH^+) 299.0. Found, %: N 9.46. $C_{12}H_{14}N_2O_3S_2$. Calculated, %: N 9.39. M.w. 298.38.

Ethyl 3-butyl-5-methyl-4-oxo-2-thioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-6-carboxylate (2c). M.p. – 239-240°C. Yield – 86%. ^{1}H NMR (*DMSO-d_6*): 0.90 (3H, t, CH_3); 1.18-1.40 (5H, m, CH_2+ $OCH_2C\underline{H}_3$); 1.60 (2H, m, CH_2); 2.70 (3H, s, CH_3); 4.17-4.34 (4H, m, $2CH_2$); 13.71 (1H, s, NH). LC/MS: m/z (MH^+) 327.2. Found, %: N 8.79. $C_{14}H_{18}N_2O_3S_2$. Calculated, %: N 8.58. M.w. 326.44.

The general method for the synthesis of compounds 5 and 6. To 0.5 mmole of thione **2** in 3.5 ml of DMF add 0.5 mmole of the corresponding alkylating agent **3** or **4** and 0.55 mmole of triethylamine. Stir the reaction mixture at 50-60°C for 3-4 h. Then after cooling dilute the reaction mixture with water, filter the precipitate formed and crystallize from ethanol.

The study of the antimicrobial activity

The study of the antimicrobial activity of the compounds synthesized was performed at the premises of the Laboratory of Biochemistry of Microorganisms and Culture Media at the State Institution "Institute of Microbiology and Immunology named after I.I.Mechnikov of the National Academy of Medical Sciences of Ukraine". According to the WHO recommendations [4, 5] to estimate the activity of the compounds tested the following strains of microorganisms were 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 inoculum suspension was prepared using a Densi-La-Meter apparatus (made by PLIVA-Lachema, Czech Republic; with the wavelength of 540 nm). The cultures were synchronized using low temperature conditions (4°C). The inoculum density was 10^7 cells per 1 ml of the medium and was determined by comparing with McFarland standard. The 18 to 24-hour old culture of the microorganism was used for the test. Mueller-Hinton agar was used ("HIMedia Laboratories Pvt. Ltd., India") for bacteria. The strain of *Candida albicans* was cultivated using Sabouraud agar ("HIMedia Laboratories Pvt. Ltd., India"). The compounds studied were introduced as DMSO solution in the concentration of 100 μg/ml with the volume of 0.3 ml.

Results and Discussion

To simplify the reaction conditions in order to improve the method the solvent with a low boiling point – 2-propanol was chosen. With the aim to control the concentration of volatile aliphatic amines the excess of

Table 1

Physico-chemical properties of ethyl 3-alkyl-2-(alkylthio)-5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxylates **5** and **6**

Comp.	Alk	Ar	Mol. formula M.w.	Yield, %, alkylation step	M.p., °C	N% calc. / found
5a	$-CH_3$	$-C_6H_5$	$C_{19}H_{19}N_3O_4S_2$ 417.51	82	235-236	10.06 / 10.15
5b	$-CH_3$	$-C_6H_4-CH_3$	$C_{20}H_{21}N_3O_4S_2$ 431.54	84	205-206	9.74 / 9.91
5c	$-CH_3$	$-C_6H_4-CH(CH_3)_2$	$C_{22}H_{25}N_3O_4S_2$ 459.59	76	208-209	9.14 / 9.27
5d	$-CH_3$	$-2-CH_3-5-Cl-C_6H_3$	$C_{20}H_{20}ClN_3O_4S_2$ 465.98	85	236	9.02 / 9.08
5e	$-C_2H_5$	$-C_6H_5$	$C_{20}H_{21}N_3O_4S_2$ 431.54	89	202	9.74 / 9.83
5f	$-C_2H_5$	$-C_6H_4-CH_3$	$C_{21}H_{23}N_3O_4S_2$ 445.56	93	202-203	9.43 / 9.58
5g	$-C_2H_5$	$-C_6H_4-CH(CH_3)_2$	$C_{23}H_{27}N_3O_4S_2$ 473.62	81	199-201	8.87 / 9.02
5h	$-C_2H_5$	$-2-CH_3-5-Cl-C_6H_3$	$C_{21}H_{22}ClN_3O_4S_2$ 480.01	73	240-242	8.75 / 8.80
5i	$-n-C_4H_9$	$-C_6H_5$	$C_{22}H_{25}N_3O_4S_2$ 459.59	91	209-210	9.14 / 9.37
5g	$-n-C_4H_9$	$-C_6H_4-CH_3$	$C_{23}H_{27}N_3O_4S_2$ 473.62	82	207-208	8.87 / 8.92
5k	$-n-C_4H_9$	$-C_6H_4-CH(CH_3)_2$	$C_{25}H_{31}N_3O_4S_2$ 501.67	78	202-203	8.38 / 8.46
5l	$-n-C_4H_9$	$-2-CH_3-5-Cl-C_6H_3$	$C_{23}H_{26}ClN_3O_4S_2$ 508.06	86	229-230	8.27 / 8.38
6a	$-CH_3$	$-C_6H_4-CH_3$	$C_{21}H_{20}N_4O_4S_2$ 456.55	71	159-160	12.27 / 12.32
6b	$-CH_3$	$-C_6H_4-Cl$	$C_{20}H_{17}ClN_4O_4S_2$ 476.96	77	184-185	11.75 / 11.90
6c	$-C_2H_5$	$-C_6H_4-CH_3$	$C_{22}H_{22}N_4O_4S_2$ 470.57	63	154-155	11.91 / 12.05
6d	$-C_2H_5$	$-C_6H_4-Cl$	$C_{21}H_{19}ClN_4O_4S_2$ 490.99	76	161-162	11.41 / 11.56
6e	$-n-C_4H_9$	$-C_6H_4-CH_3$	$C_{24}H_{26}N_4O_4S_2$ 498.63	89	157-159	11.24 / 11.43
6f	$-n-C_4H_9$	$-C_6H_4-Cl$	$C_{23}H_{23}ClN_4O_4S_2$ 519.05	79	145-147	10.79 / 10.80

these reagents was used. It has been found that in such conditions the reaction begins homogeneously and in 1.5-2 h a white precipitate is formed. For complete isolation of the product the cool reaction mixture was diluted with water, and the neutral pH value was adjusted by acidification. The crystals of the products **2** were filtered and washed with 2-propanol (Scheme).

In order to enlarge the chemical diversity compounds **2a-c** were alkylated with chloroacetamides (**3**) and 3-aryl-5-(chloromethyl)-1,2,4-oxadiazoles (**4**) (DMF-triethylamine). As the result of modification the series of S-alkyl derivatives **5** and **6** were obtained (Tab. 1).

In the ^{1}H NMR spectra of the given derivatives **5** and **6** two signals of the carbethoxy group are observed in the range of 1.27-1.30 ppm (3H, t) and 4.24-4.29 (2H, q); in some cases the signal of CH_3 ($COOC_2H_5$) overlaps with the signal of the ethyl radical at position 3 of the thieno[2,3-d]pyrimidine system, while the signal of OCH_2 may be together with SCH_2 protons peak. The spectra of all compounds **5** and **6** also contain the signal of the thiophene ring methyl group at 2.72-2.80 ppm, for compounds **5** the signal of acetamide NH protons in the range of 9.79-10.40 ppm, which position much depends upon the character of the benzene ring substituents, is typical. The signals of SCH_2 are observed in the region of 4.22-4.29 ppm for compounds **5** and strongly shifted downfield for compounds **6** to 4.89-4.93 ppm (Tab. 2).

The results of the antimicrobial activity screening for the series of compounds **5** and **6** allowed determining their wide range, but the moderate antibacterial activity

Table 2

^{1}H NMR spectral data for ethyl 3-alkyl-2-(alkylthio)-5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxylates **5** and **6**

Comp.	Chemical shift, δ, ppm			
	CH_3 thiophene (3H, s)	NH (1H, br.s)	Aliphatic protons	Aromatic protons
5a	2.79	10.38	1.29 (3H, t, $OCH_2C\underline{H}_3$); 3.50 (3H, s, NCH_3); 4.27 (4H, m, SCH_2 + $OC\underline{H}_2CH_3$);	7.07 (1H, t, H-4′); 7.32 (2H, t, H-3′ + H-5′); 7.59 (2H, d, H-2′ + H-6′)
5b	2.79	10.31	1.29 (3H, t, $OCH_2C\underline{H}_3$); 2.25 (3H, s, $ArCH_3$); 3.50 (3H, s, NCH_3); 4.22 (2H, s, SCH_2); 4.28 (2H, q, $OC\underline{H}_2CH_3$);	7.12 (2H, d, H-3′ + H-5′); 7.46 (2H, d, H-2′ + H-6′)
5c	2.78	10.29	1.17 (6H, d, $2CH_3$); 1.29 (3H, t, $OCH_2C\underline{H}_3$); 2.83 (1H, m, $C\underline{H}(CH_3)_2$); 3.50 (3H, s, NCH_3); 4.22 (2H, s, SCH_2); 4.28 (2H, q, $OC\underline{H}_2CH_3$);	7.18 (2H, d, H-3′ + H-5′); 7.48 (2H, d, H-2′ + H-6′)
5d	2.80	9.82	1.30 (3H, t, $OCH_2C\underline{H}_3$); 2.23 (3H, s, $ArCH_3$); 3.51 (3H, s, NCH_3); 4.29 (4H, m, SCH_2 + $OC\underline{H}_2CH_3$);	7.16 (1H, d, H-3′); 7.26 (1H, d, H-4′); 7.50 (1H, s, H-6′);
5e	2.80	10.40	1.29 (6H, m, $OCH_2C\underline{H}_3$ + $NCH_2C\underline{H}_3$); 4.11 (2H, q, $NC\underline{H}_2CH_3$); 4.25 (2H, s, SCH_2); 4.28 (2H, q, $OC\underline{H}_2CH_3$);	7.07 (1H, m, H-4′); 7.32 (2H, t, H-3′ + H-5′); 7.59 (2H, d, H-2′ + H-6′)
5f	2.80	10.25	1.30 (6H, t, $OCH_2C\underline{H}_3$ + $NCH_2C\underline{H}_3$); 2.26 (3H, s, $ArCH_3$); 4.11 (2H, q, $NC\underline{H}_2CH_3$); 4.22 (2H, s, SCH_2); 4.29 (2H, q, $OC\underline{H}_2CH_3$);	7.12 (2H, d, H-3′ + H-5′); 7.46 (2H, d, H-2′ + H-6′)
5g	2.80	10.30	1.18 (6H, d, $2CH_3$); 1.29 (6H, t, $OCH_2C\underline{H}_3$ + $NCH_2C\underline{H}_3$); 2.83 (1H, m, $C\underline{H}(CH_3)_2$); 4.11 (2H, q, $NC\underline{H}_2CH_3$); 4.23 (2H, s, SCH_2); 4.28 (2H, q, $OC\underline{H}_2CH_3$);	7.18 (2H, d, H-3′ + H-5′); 7.48 (2H, d, H-2′ + H-6′)
5h	2.80	9.83	1.30 (6H, t, $OCH_2C\underline{H}_3$ + $NCH_2C\underline{H}_3$); 2.23 (3H, s, $ArCH_3$); 4.11 (2H, q, $NC\underline{H}_2CH_3$); 4.28 (4H, m, SCH_2 + $OC\underline{H}_2CH_3$);	7.16 (1H, d, H-3′); 7.26 (1H, d, H-4′); 7.50 (1H, s, H-6′);
5i	2.79	10.40	0.96 (3H, m, CH_3); 1.29 (3H, m, $OCH_2C\underline{H}_3$); 1.40 (2H, m, CH_2); 1.68 (2H, m, CH_2); 4.04 (2H, m, $NC\underline{H}_2C_3H_7$); 4.24 (4H, m, SCH_2 + $OC\underline{H}_2CH_3$);	7.07 (1H, m, H-4′); 7.32 (2H, m, H-3′ + H-5′); 7.58 (2H, m, H-2′ + H-6′)
5j	2.79	10.28	0.95 (3H, t, CH_3); 1.29 (3H, m, $OCH_2C\underline{H}_3$); 1.40 (2H, m, CH_2); 1.69 (2H, m, CH_2); 2.25 (3H, s, $ArCH_3$); 4.04 (2H, m, $NC\underline{H}_2C_3H_7$); 4.22 (2H, s, SCH_2); 4.28 (2H, q, $OC\underline{H}_2CH_3$);	7.12 (2H, d, H-3′ + H-5′); 7.46 (2H, d, H-2′ + H-6′)
5k	2.79	10.32	0.95 (3H, t, CH_3); 1.17 (6H, d, $2CH_3$); 1.29 (3H, m, $OCH_2C\underline{H}_3$); 1.40 (2H, q, CH_2); 1.68 (2H, m, CH_2); 2.83 (1H, m, $C\underline{H}(CH_3)_2$); 4.04 (2H, m, $NC\underline{H}_2C_3H_7$); 4.22 (2H, s, SCH_2); 4.28 (2H, q, $OC\underline{H}_2CH_3$);	7.18 (2H, d, H-3′ + H-5′); 7.48 (2H, d, H-2′ + H-6′)
5l	2.80	9.79	0.95 (3H, t, CH_3); 1.30 (3H, t, $OCH_2C\underline{H}_3$); 1.40 (2H, q, CH_2); 1.69 (2H, m, CH_2); 2.23 (3H, s, $ArCH_3$); 4.05 (2H, m, $NC\underline{H}_2C_3H_7$); 4.29 (4H, m, SCH_2 + $OC\underline{H}_2CH_3$);	7.15 (1H, d, H-3′); 7.25 (1H, d, H-4′); 7.51 (1H, s, H-6′);
6a	2.72	–	1.29 (3H, t, $OCH_2C\underline{H}_3$); 2.35 (3H, s, CH_3); 3.47 (3H, s, NCH_3); 4.25 (2H, q, $OC\underline{H}_2CH_3$); 4.89 (2H, s, SCH_2);	7.33 (2H, d, H-2′ + H-6′); 7.85 (2H, d, H-3′ + H-5′)
6b	2.72	–	1.28 (3H, t, $OCH_2C\underline{H}_3$); 3.48 (3H, s, NCH_3); 4.25 (2H, q, $OC\underline{H}_2CH_3$); 4.91 (2H, s, SCH_2);	7.60 (2H, d, H-2′ + H-6′); 7.97 (2H, d, H-3′ + H-5′)
6c	2.75	–	1.29 (6H, m, $OCH_2C\underline{H}_3$ + $NCH_2C\underline{H}_3$); 2.33 (3H, s, CH_3); 4.08 (2H, q, $NC\underline{H}_2CH_3$); 4.27 (2H, q, $OC\underline{H}_2CH_3$); 4.91 (2H, s, SCH_2);	7.34 (2H, d, H-2′ + H-6′); 7.86 (2H, d, H-3′ + H-5′)
6d	2.76	–	1.29 (6H, m, $OCH_2C\underline{H}_3$ + $NCH_2C\underline{H}_3$); 4.08 (2H, q, $NC\underline{H}_2CH_3$); 4.27 (2H, q, $OC\underline{H}_2CH_3$); 4.93 (2H, s, SCH_2);	7.61 (2H, d, H-2′ + H-6′); 7.98 (2H, d, H-3′ + H-5′)
6e	2.76	–	0.95 (3H, t, CH_3); 1.28 (3H, t, $OCH_2C\underline{H}_3$); 1.39 (2H, q, CH_2); 1.67 (2H, m, CH_2); 2.37 (3H, s, $ArCH_3$); 4.02 (2H, m, $NC\underline{H}_2C_3H_7$); 4.26 (2H, q, $OC\underline{H}_2CH_3$); 4.91 (2H, s, SCH_2);	7.36 (2H, d, H-2′ + H-6′); 7.86 (2H, d, H-3′ + H-5′)
6f	2.74	–	0.95 (3H, t, CH_3); 1.27 (3H, t, $OCH_2C\underline{H}_3$); 1.40 (2H, q, CH_2); 1.67 (2H, m, CH_2); 4.01 (2H, m, $NC\underline{H}_2C_3H_7$); 4.25 (2H, q, $OC\underline{H}_2CH_3$); 4.92 (2H, s, SCH_2);	7.60 (2H, d, H-2′ + H-6′); 7.97 (2H, d, H-3′ + H-5′)

Table 3

The antimicrobial activity of ethyl 3-alkyl-2-(alkylthio)-5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxylates **5** and **6**

Comp.	Diameter of the growth inhibition zone in mm, number of experiments n=3					
	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*
5a	15, 14, 15	15, 15, 15	growth	growth	17, 16, 17	18,18, 19
5b	15, 16, 16	16, 15, 14	growth	13, 14, 15	16, 16, 17	18, 18, 18
5c	17, 18, 17	17, 17, 17	14, 15, 15	15, 14, 15	16, 17, 17	17, 16, 17
5d	16, 16, 17	17, 17, 18	14, 15, 15	15, 15, 15	18, 18, 18	16, 17, 17
5e	12, 13, 13	18, 16, 17	growth	13, 14, 13	17, 16, 17	16, 16, 17
5f	17, 17, 18	16, 17, 17	16, 16, 17	17, 17, 16	17, 18, 17	18, 17, 18
5g	17, 17, 17	16, 17, 15	16, 16, 16	16, 16, 17	19, 19, 18	18, 18, 18
5h	16, 16, 16	16, 16, 17	14, 15. 15	14, 15, 16	17, 16, 17	17, 18, 18
5i	16, 16, 16	15, 16, 16	14, 14, 14	17, 17, 18	18, 17, 18	16, 16, 17
5j	14, 13, 14	14, 14, 14	growth	15, 14, 14	16, 16, 17	20, 21, 21
5k	18, 17, 17	16, 16, 15	15, 15, 16	16, 16, 16	19, 20, 20	23, 22, 23
5l	13, 13, 14	15, 16, 16	16, 15, 16	15, 16, 16	18, 17, 18	22, 23, 23
6a	17, 18, 17	17, 16, 16	15, 15, 15	16, 17, 16	18, 19, 18	18, 17, 18
6b	16, 16, 15	16, 17, 17	16, 15, 15	16, 15, 16	17, 18, 18	16, 17, 17
6c	12, 13, 12	13, 13, 14	growth	14, 15, 15	16, 16, 17	17,17, 16
6d	18, 17, 18	17, 16, 17	15, 14, 15	16, 16, 17	18, 19, 19	13, 13, 14
6e	14, 14, 15	16, 15, 16	growth	14, 15, 15	16, 17, 17	20, 21, 20
6f	14, 14, 14	16, 16, 16	14, 13, 14	14, 15, 15	18, 18, 17	21, 22, 21
Metr.*	14, 15, 14	14, 13, 14	growth	growth	16, 15, 16	14, 14, 14
Strept.**	15, 16, 15	15, 16, 17	growth	growth	17, 16, 17	growth

* Metr. – Metronidazole, DMSO solution, with the concentration of 30 μg/ml;
** Strept. – Streptomycin, H_2O solution, with the concentration of 30 μg/ml;

for the most of the compounds tested, being similar to the reference drugs Streptomycin and Metronidazole. The most active compounds with *n*-butyl substituent in position 3 of the thieno[2,3-d]pyrimidine system **5j-5l** and **6e,f**, were found to inhibit the growth of *Candida albicans* (Tab. 3).

CONCLUSIONS

A novel and effective method for the synthesis of ethyl 3-alkyl-5-methyl-4-oxo-2-thioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-6-carboxylate derivatives has been developed with further modification of these compounds to obtain 2-alkylthio derivatives. The study of the antimicrobial activity of the final products has allowed to determine their moderate antibacterial activity though some compounds containing the *n*-butyl substituent in position 3 of the thieno[2,3-d]pyrimidine system significantly inhibit the growth of *Candida albicans* fungi.

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СИНТЕЗ ТА АНТИМІКРОБНА АКТИВНІСТЬ НОВИХ ПОХІДНИХ ЕТИЛ 3-АЛКІЛ-2-(АЛКІЛТІО)-5-МЕТИЛ-4-ОКСО-3,4-ДИГІДРОТІЄНО[2,3-*d*]ПІРИМІДИН-6-КАРБОКСИЛАТІВ

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Ключові слова: *тіофен; піримідин; меркаптани; алкілування; циклізація*

Розроблена ефективна методика одержання похідних етил 3-алкіл-5-метил-4-оксо-2-тіоксо-1,2,3,4-тетрагідротієно[2,3-d]піримідин-6-карбоксилатів шляхом проведення взаємодії діетил 3-метил-5-{[(метилсульфаніл)карботіоїл]аміно}тіофен-2,4-дикарбоксилату з нижчими аліфатичними амінами у середовищі 2-пропанолу. Такі умови реакції дозволяють легко виділяти бажані сполуки та значно покращують виходи цільових тіонів. Подальшу модифікацію етил 3-акліл-5-метил-4-оксо-2-тіоксо-1,2,3,4-тетрагідротієно[2,3-d]піримідин-6-карбоксилатів проводили шляхом взаємодії з хлороацетамідами та 3-арил-5-(хлорометил)-1,2,4-оксадіазолами (ДМФА-триетиламін). Будову отриманих сполук було підтверджено даними ЯМР-спектроскопії; для продуктів алкілування сигнали протонів карбетокси-групи проявляються у вигляді двох сигналів у діапазоні 1.27-1.30 м.ч. (3Н, т) та 4.24-4.29 (2Н, кв), а протони SCH_2 дають сигнал у діапазоні 4.22-4.93 м.ч. Дослідження антимікробної активності продуктів алкілування отриманих фунціоналізованих похідних тієно[2,3-d]піримідину, відповідно етил 3-алкіл-5-метил-2-({2-[ариламіно]-2-оксоетил}тіо)-4-оксо-3,4-дигідротієно[2,3-d]піримідин-6-карбоксилатів та етил 3-алкіл-5-метил-4-оксо-2-{[(3-арил-1,2,4-оксадіазол-5-іл)метил]тіо}-3,4-дигідротієно[2,3-d]піримідин-6-карбоксилатів дозволило встановити, що сполуки чинять помірну антибактеріальну активність, проте похідні з н-бутильним замісником у положенні 3 тієно[2,3-d]піримідинової системи значно пригнічують ріст грибів Candida albicans.

СИНТЕЗ И ПРОТИВОМИКРОБНАЯ АКТИВНОСТЬ НОВЫХ ПРОИЗВОДНЫХ ЭТИЛ 3-АЛКИЛ-2-(АЛКИЛТИО)-5-МЕТИЛ-4-ОКСО-3,4-ДИГИДРОТИЕНО[2,3-*d*]ПИРИМИДИН-6-КАРБОКСИЛАТОВ

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Ключевые слова: *тиофен; пиримидин; меркаптаны; алкилирование; циклизация*

Разработана эффективная методика получения производных этил 3-алкил-5-метил-4-оксо-2-тиоксо-1,2,3,4-тетрагидротиено[2,3-d]пиримидин-6-карбоксилатов путем проведения взаимодействия диэтил 3-метил-5-{[(метилсульфанил)карботиоил]амино}тиофен-2,4-дикарбоксилата с низшими алифатическими аминами в среде 2-пропанола. Такие условия реакции позволяют легко выделять целевые соединения и значительно улучшают выходы целевых тионов. Дальнейшую модификацию этил 3-алкил-5-метил-4-оксо-2-тиоксо-1,2,3,4-тетрагидротиено[2,3-d]пиримидин-6-карбоксилатов проводили путем взаимодействия с хлорацетамидами и 3-арил-5-(хлорометил)-1,2,4-оксадиазолами (ДМФА-триэтиламин). Строение полученных соединений было подтверждено данными ЯМР-спектроскопии; для продуктов алкилирования сигналы протонов карбэтокси-группы проявляются в виде двух сигналов в диапазоне 1.27-1.30 м.д. (3Н, т) и 4.24-4.29 (2Н, кв), а протоны SCH_2 дают сигнал в диапазоне 4.22-4.93 м.д. Исследования противомикробной активности продуктов алкилирования полученных функционализированных производных тиено[2,3-d]пиримидина, соответственно этил 3-алкил-5-метил-2-({2-[ариламино]-2-оксоэтил}тио)-4-оксо-3,4-дигидротиено[2,3-d] пиримидин-6-карбоксилатов и этил 3-алкил-5-метил-4-оксо-2-{[(3-арил-1,2,4-оксадиазол-5-ил) метил]тио}-3,4-дигидротиено[2,3-d]пиримидин-6-карбоксилатов позволило установить, что соединения проявляют умеренную антибактериальную активность, однако производные с н-бутильным заместителем в положении 3 тиено[2,3-d]пиримидиновой системы значительно угнетают рост грибов Candida albicans.

Recommended by Doctor of Chemistry, professor I.O.Zhuravel

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HYDROLYTIC CLEAVAGE OF THE PYRIMIDINE RING IN 2-ARYL-[1,2,4]TRIAZOLE[1,5-*c*]QUINAZOLINES: PHYSICO-CHEMICAL PROPERTIES AND THE HYPOGLYCEMIC ACTIVITY OF THE COMPOUNDS SYNTHESIZED

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Key words: 2-aryl-[1,2,4]triazolo[1,5-c]quinazolines; hydrolytic cleavage; hypoglycemic activity

It has been shown that 2-aryl-[1,2,4]triazolo[1,5-c]-quinazolines under the action of nucleophilic agents (hydrazine hydrate, sodium hydroxide, sodium methoxide, hydrochloric acid) undergo hydrolytic cleavage followed by formation of [2-(3-aryl-1H-1,2,4-triazol-5-yl)-phenyl]amines. The rational synthetic protocols for the compounds mentioned above, namely heating in the hydrochloric acid solution at 90-95°C for 60 min, have been proposed. It has been found that substituents in position 2 of the triazoloquinazoline moiety do not significantly affect duration of the reaction and the yields of products. Purity and the structure of the compounds synthesized have been proven by the corresponding physico-chemical methods, namely: elemental analysis, LC-MS, ^{1}H, ^{13}C NMR-spectrometry and X-ray structural study. The azole-azole prototropic tautomery has been substantiated using physico-chemical analytical methods. According to the data obtained in gas or DMSO medium compounds 2 exist as tautomer A or C, while in the crystal lattice the anilines mentioned exist as A-form. It has been determined that 2-(3-aryl-1H-1,2,4-triazol-5-yl)phenyl]amines (2.1, 2.8, 2.14) in the dose of 10 mg/kg are as good as reference-drugs Metformin (in the doses of 50 and 200 mg/kg) and Gliclazide (in the dose of 50 mg/kg) by their hypoglycemic activity when assessing specific pharmacological activities in oral glucose tolerance test (OGTT), rapid insulin and adrenaline test models.

Recent publication describes hydrolytic cleavage of azole and azine condensed cycles under the action of nucleophiles [1, 3, 6, 4, 9, 11, 12, 15, 17, 20, 25, 26, 28, 29]. It is known that kinetics of the reactions mentioned depends on basic properties of nucleophilic reagents. Strong nucleophiles (hydroxides and alkoxides of alkaline metals, hydrazine hydrate) easily react with the given substrates in the water or alcohol-water medium for 1 h and form products with high yields [4, 15, 20, 25, 26, 28, 29]. Moreover, water may play the role of a nucleophile in hydrolytic cleavage reactions. In this case the reaction proceeds for 1 h and needs acidic catalysis [1, 6]. It has been noted that cleavage of the pyridine cycle of 2-aryl-[1,2,4]triazolo[1,5-*c*]quinazoline is insufficiently known in spite of potentially bioactive products of this transformation. In addition, products of the reactions mentioned may take a worth place in the process of development of approaches for forming new heterocyclic systems and drug discovery [2, 8, 13, 18, 19, 23, 24].

Moreover, we found reports where the hypoglycemic activity of derivatives of 1,2,4-triazole were described. Thus, A.K.Mohammed Iqbal and co-authors discussed in their research the hypoglycemic and hypolipidemic action of novel compounds, which molecules contained the thiazolidone fragment attached to the triazole cycle via phenoxyethenthiol "linker" groups [14]. 4-Methyl-3-(R-phenyl-,1-methyl-1H-indol-4(5)-yl)-5-(R-phenyl-)-4*H*-1,2,4-triazoles as selective inhibitors of 11β-hydroxysteroid dehydrogenase 1 were described as prospective glucose-lowering agents by Susan D. Aster and co-authors [7], and "ELI LILLY and COMPANY" applied for patent series of 2-R_1-4-R_2-5-alkaryl-(alkheteryl-, aryl-(heteryl-)oxy-, aryl-(heteryl-)thio-, aryl-(heteryl-)amino-)-2,4-dihydro-3*H*-1,2,4-triazol-3-ones(thiones) with high affinity to nuclear hormonal receptors. They may be used for treating diabetes, cardiovascular disorders, obesity, X-syndrome and gastrointestinal disorders [21, 22], etc.

Thus, the aims of the present research were to study the peculiarities of the given type of the reaction, elucidation of the effect of *o*-, *m*-, *p*-substituted aryl moiety in position 2 of [1,2,4]triazolo[1,5-*c*]quinazolines on the process of the pyrimidine cycle hydrolytic cleavage, as well as the hypoglycemic action of the compounds synthesized.

Experimental Part

1. Chemistry

1.1. General method. Melting points were determined in open capillary tubes and were uncorrected. The ele-

mental analyses (C, H, N, S) were performed using an ELEMENTAR vario EL Cube analyzer (USA). Analyses were indicated by the symbols of the elements or functions within ±0.3% of the theoretical values. ^{1}H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz): were recorded on a Varian-Mercury 400 (Varian Inc., Palo Alto, CA, USA) spectrometer with TMS as an internal standard in DMSO-d_6 solution. LC-MS were recorded using the chromatography/mass spectrometric system consisting of high performance liquid chromatographer "Agilent 1100 Series" (Agilent, Palo Alto, CA, USA) equipped with a diode-matrix and a mass-selective detector "Agilent LC/MSD SL" (atmospheric pressure chemical ionization – APCI). Electron impact mass spectra (EI-MS) were recorded on a Varian 1200 L instrument at 70 eV (Varian, USA).

Substances 1.1-1.16 were synthesized according to the procedures reported [16]. Other starting materials and solvents were obtained from commercially available sources and used without additional purification.

1.2. The general procedure for the synthesis of [2-(3-aryl-1H-1,2,4-triazole-5-yl)penyl]amines (2.1-2.17). Add 1-2 ml of concentrated hydrochloric acid to 10 mmol of 2-aryl-[1,2,4]triazole[1,5-*c*]quinazolines (1.1-1.17) in 10 ml of the water-alcohol mixture (1:1). Reflux the mixture obtained for 60 min. Then dilute the mixture with water and add 5% solution of sodium acetate to form the solution with pH 5-6. Filter the precipitates of compounds 2.1-2.17 and dry. Crystallize the compounds from methanol.

[2-(3-Phenyl-1*H*-1,2,4-triazole-5-yl)phenyl]amine (2.1). EI-MC, *m/z* (I_{rel}, %): 237(15.6), 236($M^{+\bullet}$. 100), 207(6.0), 119(9.7), 118(21.7), 105(7.0), 104(38.6), 103 (4.0), 91(17.4), 90(7.2), 78(9.0), 77(27.1),63(5.1), 51(9.1).

{2-[3-(2-Bromophenyl)-1*H*-1,2,4-triazole-5-yl]phenyl}amine (2.4). EI-MC, *m/z* (I_{rel}, %): 317(13.2), 316(97.7), 315(12.9), 314($M^{+\bullet}$. 100), 236(7.4), 198(8.3), 196(9.6), 133(7.2), 131(5.7), 129(8.8), 119(5.8), 118(14.1), 117(6.7), 106(5.5), 105(11.8), 104(92.1), 103(31.2), 102(17.4), 91(14.5), 90(21.8), 89(14.0), 88(7.3), 85(5.4), 79(7.1), 78(22.5), 77(38.3), 76(13.1), 75(8.0), 65(9.2), 64(7.9), 63(11.6), 62(6.1), 52(7.0), 51(15.5).

{2-[3-(2-Methoxyphenyl)-1*H*-1,2,4-triazole-5-yl]phenyl}amine (2.6). EI-MC, *m/z* (I_{rel}, %): 267(16.9), 266($M^{+\bullet}$. 100), 265(8.2), 252(8.1), 248(6.3), 237(6.7), 236(9.3), 223(10.0) 146(9.3), 133(6.6), 132(5.5), 119(24.3), 118(35.9), 105(19.6), 104(48.6), 103(5.8), 102(5.1), 92(5.8), 91(19.1), 90(9.2), 85(9.1), 83(11.8), 79(7.4), 78(16.1), 77(38.8), 76(6.6), 65(8.6), 64(6.1), 63(6.4), 51(15.5).

{2-[3-(4-Methylphenyl)-1*H*-1,2,4-triazole-5-yl]phenyl}amine (2.12). EI-MC, *m/z* (I_{rel}, %): 251(16.3), 250($M^{+\bullet}$. 100), 221(7.8), 133(7.3), 132(18.8), 131(11.8), 119 (1.8), 118(7.2), 105(9.9), 104(49.6), 91(20.3), 90(5.3), 78(12.4), 77(18.5), 65(7.5), 51(7.7); EI-MC. *m/z* (I_{rel}. %) = 267(18.5), 266($M^{+\bullet}$. 100), 223(7.0), 148(10.5), 133(30.5), 119(5.6), 118(5.2), 105(17.9), 104(40.5), 91(8.1), 90(5.8), 78(12.6), 77(16.3), 76(5.7), 65(5.4), 63(5.1), 51(9.4).

{2-[3-(4-Fluorophenyl)-1*H*-1,2,4-triazole-5-yl]phenyl}amine (2.13). EI-MC, *m/z* (I_{rel}, %) = 255(15,0), 254($M^{+\bullet}$, 100), 137(9,5), 136(19,6), 109(18,4), 105(8,0), 104(36,9), 95(10,1), 78(9,3), 77(13,2), 51(6,8).

{2-[3-(4-Chlorophenyl)-1*H*-1,2,4-triazole-5-yl]phenyl}amine (2.14). EI-MC, *m/z* (I_{rel}, %): 272(37.9), 271(14.1), 270($M^{+\bullet}$. 100), 154(9.6), 153(9.3), 152(21.8), 129(4.4), 127(5.3), 125(15.7), 119(7.2), 118(8.3), 111(11.2), 105(12.6), 104(63.4), 103(8.6), 102(7.5), 91(10), 90(19.1), 89(7.6), 85(8.3), 83(6.3), 79(6.6), 78(17.1), 77(26.6), 76(8.3), 75(10.7), 71(5.5), 69(9.4), 65(6.2), 63(9.8), 57(8.1), 55(6.2), 52(6.2), 51(10.8).

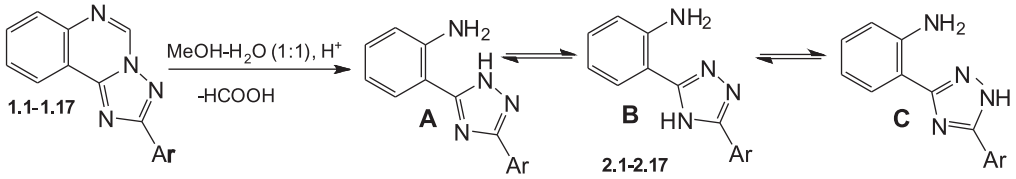
(2-{3-[4-(Trifluoromethyl)phenyl]-1*H*-1,2,4-triazole-5-yl]phenyl}amine (2.16). EI-MC, *m/z* (I_{rel}, %) = 305(17.5), 304($M^{+\bullet}$. 100), 187(10.1), 186(10.7), 145(9.5), 119(8.3), 118(11.2), 105(9.0), 104(51.4), 78(13.4), 77(20.0), 65(5.2), 51(10.2).

{2-[3-(4-Methoxyphenyl)-1*H*-1,2,4-triazole-5-yl]phenyl}amine (2.17). EI-MC, *m/z* (I_{rel}, %): 267(18.5), 266($M^{+\bullet}$. 100), 223(7.0), 148(10.5), 133(30.5), 119(5.6), 118(5.2), 105(17.9), 104(40.5), 91(8.1), 90(5.8), 78(12.6), 77(16.3), 76(5.7), 65(5.4), 63(5.1), 51(9.4).

1.3. X-Ray diffraction study of **2.16**. The colourless crystals of 2.16 ($C_{15}H_{11}N_4F_3$) are orthorhombic. At 293 K a = 7.745(2), b = 11.435(2), c = 30.528(7) Å, V = 2704(1) Å^3, Mr = 304.28, Z = 8, space group Pbca, d_{calc} = 1.495 g/cm^3, $\mu(MoK_\alpha)$ = 0.122 mm^{-1}, F(000) = 1248. Intensities of 16607 reflections (2380 independent, R_{int}=0.194) were measured on a "Xcalibur-3" diffractometer (graphite monochromated MoK_α radiation, CCD detector, ω-scaning, 2Θmax = 50°). The structure was solved by the direct method using SHELXTL package [27]. Positions of the hydrogen atoms were located from electron density difference maps and refined by the "riding" model with $U_{iso} = 1.2U_{eq}$ of the carrier atom. The hydrogen atoms of the amino and NH groups were refined in isotropic approximation. Full-matrix least-squares refinement against F^2 in anisotropic approximation for non-hydrogen atoms using 2354 reflections was converged to wR_2 = 0.191 (R_1 = 0.068 for 927 reflections with $F>4\sigma(F)$, S = 0.869). The final atomic coordinates and crystallographic data for molecule 2.16 were deposited from the Cambridge Crystallographic Data Centre, 12 Union Road, CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk). They are available on request quoting the deposition numbers CCDC 1029407.

2. Pharmacology

2.1. Hypoglycemia activity test. The study of the hypoglycemic action was conducted using 120 male Wistar white rats (with the weight of 260-280 g., aged 3.5 months) from nursery of PE "Biomodelservice" (Kyiv, Ukraine). Experiments on animals were done according to bioethic principles [10]. Selected after quarantine the animals were divided by random sampling in groups of 6 male rats on the assumption of the absence of the external signs of diseases and homogeneity by weight (±15%). Experimental animals were not fed within 12 h before introduction of the compounds studied. The weight of all animals was measured before the experiments. The compounds studied were injected intragastrically using atraumatic probe as the water solution or a finely dispersed suspension stabilized by Tween 80 in the dose of 10 mg/kg. Intact and control groups received equivalent volumes of water by the same way. The hypoglycemic



Ar = C_6H_5, 2-FC_6H_4, 2-ClC_6H_4, 2-BrC_6H_4, 2-$CF_3C_6H_4$, 2-$CH_3OC_6H_4$, 3-FC_6H_4, 3-ClC_6H_4, 3-BrC_6H_4, 3-$CF_3C_6H_4$, 3-$CH_3OC_6H_4$, 4-$CH_3C_6H_4$, 4-FC_6H_4, 4-ClC_6H_4, 4-BrC_6H_4, 4-$CF_3C_6H_4$, 4-$CH_3OC_6H_4$

Scheme. Hydrolytic cleavage of the pyrimidine ring in 2-aryl-[1,2,4]triazolo[1,5-*c*]-quinazolines and tautomeric transformation of [2-(3-aryl[1,2,4]triazol-5-yl)phenyl]amines.

action of the compounds synthesized was evaluated by changes in the glucose level before and after injection of the compounds studied. Measurements of the glucose level were carried out in 2, 4, 6 and 8 h after injection.

The primary insulin resistance was induced by a daily intramuscular injection of glucocorticoid, namely dexamethasone, in the dose of 0.125 mg/kg for 13 days [5, 30]. The state of glucose homeostasis was evaluated by values of basal glycemia and carbohydrate tolerance determined by the oral test for glucose tolerance, rapid insulin test and adrenaline test [5, 30]. Metformin in the doses of 50 and 200 mg/kg and Gliclazide in the dose of 50 mg/kg were used as reference drugs.

Statistical analysis was performed using a standard software complex, namely "Microsoft Office Excel 2003" and "STATISTICA® for Windows 6.0" (StatSoft Inc., № AXXR712D833214FAN5). For each estimated value in the arithmetic mean (M), and in the standard error of the mean (±m) were determined. During verification of statistical hypotheses, in the null hypothesis was rejected if in the statistical criterion $p<0.05$.

Results and Discussion

1. *Chemistry.* It was found that hydrolytic cleavage of 2-aryl-[1,2,4]triazolo[1,5-*c*]-quinazolines (1.1-1.17) occurred in the alcohol-water mixture medium in the presence of hydrochloric acid at 90-95°C for 60 min with the yields of 89.6-99.2% (Scheme). The synthetic protocols proposed are optimal for preparation of target compounds, while using hydrazine hydrate, sodium hydroxide and sodium methylate as reagents resulted not only in 2.1-2.17, but in products of greater decomposition of the initial molecule. Increase of the process time did not affect the yields of 2.1-2.17. As we considered, protonation of N_6-atom, which caused increase of the positive charge at C_5-atom, was significant for hydrolytic cleavage process. An adduct formed easily added the molecule of water (nucleophile), and it resulted in cleavage of N(4)–C(5) bond, and followed by the nucleophilic attack of another molecule of water leading to elimination of formic acid and formation of 2.1-2.17. We also noted that in the conditions proposed there was no significant effect of the substituent's nature in position 2 of the triazoloquinazoline system on duration of the reaction and yields of final products.

Purity and the structure of the compounds synthesized were proven by the corresponding physico-chemical methods, namely: IR, ^{1}H and ^{13}C NMR, mass-spectrometry and X-ray structural analysis. In LC-MS spectra of 2.1-2.17 the high-intensive signals of quasi-molecular ions [M+1] and [M+2] corresponding to the calculated molecular weight were observed and definitely proved the fact of the pyrimidine cycle cleavage of the pyrimidine fragment in 1.1-1.17 (Tab. 1).

In ^{1}H NMR-spectra of compounds 2.1-2.17 the signals of aromatic protons of the aniline fragment with the proper chemical shift and multiplicity were observed, namely: doublet H^3 at 8.11-7.78 ppm, triplet H^4 – 6.96-6.51 ppm, triplet H^5 – 7.57-7.08 ppm, doublet H^6 – 7.16-6.74 ppm (Table 2). Chemical shifts of the aryl fragment in position 3 depended on the position and the nature of substituents and were registered at 8.41-7.02 ppm. Among the peculiarities of ^{1}H NMR-spectra for 2.1-2.17 the signals caused by the presence of NH_2-group were observed. Thus, the signals mentioned were present as a broad singlet at 6.75-6.26 ppm (2.1-2.3, 2.6-2.9, 2.12, 2.14, 2.16) or absent (2.4, 2.5, 2.10, 2.11, 2.13, 2.15, 2.17) as the result of exchange processes caused by prototropic azol-azol tautomery in 2.1-2.17 (Scheme). The absence (2.8, 2.11, 2.12, 2.14, 2.15, 2.17), doubling (2.1) or broadening (2.2-2.7, 2.9, 2.10, 2.13, 2.16) of signal caused by NH-proton of the triazole cycle may be also explained by the abovementioned phenomenon. Thus, [2-(3-R-[1,2,4]triazol-5-yl)phenyl]amines (2.1-2.17) in DMSO-d^6 are subjected to tautomery and may exist as 2H (A), 1H (C) and 4H (B) forms (Scheme).

For additional verification of the structure of the compounds synthesized, as well as for tautomeric processes ^{13}C NMR-spectra were studied (Table 3). Signal correlations were carried out using the DEPT-method. It was found that signals caused by C^3 and C^5 atoms of the triazole cycle in molecules 2.1-2.17 were observed as broad singlets at 162.81-158.10 ppm and 158.86-150.79 ppm. It proves tautomeric transitions in DMSO-d^6 solutions. Besides, the signal of the atom of the aniline fragment deshielded as a result of the electron effect of the amino group C^1 proves hydrolytic cleavage of the pyrimidine cycle.

Mass-spectra (EI) of compounds (2.1, 2.4, 2.6, 2.12, 2.13, 2.14, 2.16, 2.17) were characterized by high molecular ions $[M]^{+\bullet}$. It is important to note that a donor or acceptor affecting the aryl substituent in position 2 had a significant influence on the directions of fragmentation. Thus, for compounds containing a donor substituent (2.1, 2.6, 2.12, 2.17) two parallel directions of fragmentation were observed. The first one was caused by breaking N(2)–C(3) and C(5)–N(1) bonds followed

Table 1

The physico-chemical data of [2-(3-aryl-1*H*-1,2,4-triazol-5-yl)phenyl]amine (**2.1-2.17**)

Compd.	R	M.p.,°C	Yield, %	Calculated, N (%)	Molecular formula	Found, N (%)	LC-MS, *m/z*
2.1	H	188-190	99.2	23.71	$C_{14}H_{12}N_4$	23.73	237 [M+1], 238 [M+2]
2.2	2-F	195-197	89.6	22.04	$C_{14}H_{11}FN_4$	22.07	255 [M+1], 256 [M+2]
2.3	2-Cl	156-158	92.8	20.70	$C_{14}H_{11}ClN_4$	20.73	271 [M+1], 273 [M+3], 274 [M+4]
2.4	2-Br	143-145	92.9	17.78	$C_{14}H_{11}BrN_4$	17.75	316 [M+1], 317 [M+2]
2.5	2-CF_3	201-203	90.7	18.41	$C_{15}H_{11}F_3N_4$	18.38	305 [M+1], 306 [M+2]
2.6	2-OCH_3	135-136	90.6	21.04	$C_{15}H_{14}N_4O$	21.06	267 [M+1], 268 [M+2]
2.7	3-F	215-217	93.8	22.04	$C_{14}H_{11}FN_4$	22.01	255 [M+1], 256 [M+2]
2.8	3-Cl	196-197	95.5	20.70	$C_{14}H_{11}ClN_4$	20.67	271 [M+1], 273 [M+3], 274 [M+4]
2.9	3-Br	196-197	96.6	17.78	$C_{14}H_{11}BrN_4$	17.81	316 [M+1], 317 [M+2]
2.10	3-CF_3	237-239	94.2	18.41	$C_{15}H_{11}F_3N_4$	18.45	305 [M+1], 306 [M+2]
2.11	3-OCH_3	177-179	95.5	21.04	$C_{15}H_{14}N_4O$	21.09	267 [M+1], 268 [M+2]
2.12	4-CH_3	166-168	97.6	22.38	$C_{15}H_{14}N_4$	22.35	251 [M+1], 252 [M+2]
2.13	4-F	209-211	93.4	22.04	$C_{14}H_{11}FN_4$	22.01	255 [M+1], 256 [M+2]
2.14	4-Cl	289-291	97.3	20.70	$C_{14}H_{11}ClN_4$	20.73	271 [M+1], 273 [M+3], 274 [M+4]
2.15	4-Br	216-218	98.4	17.78	$C_{14}H_{11}BrN_4$	17.76	305 [M+1], 306 [M+2]
2.16	4-CF_3	252-253	94.1	18.41	$C_{15}H_{11}F_3N_4$	18.39	305 [M+1], 306 [M+2]
2.17	4-OCH_3	206-207	91.3	21.04	$C_{15}H_{14}N_4O$	21.01	267 [M+1], 268 [M+2]

Table 2

^{1}H NMR-spectra of [2-(3-aryl-1*H*-1,2,4-triazole-5-yl)phenyl]amine (**2.1-2.17**)

Compd.	^{1}H NMR, δ (ppm), *J* (Hz)						
	NH (bs)	H-3, (d)	H-5, (t)	H-6, (d)	NH_2 (bs)	H-4, (t)	3-Ar
2.1	14.48/14.20	7.78 (7.7)	7.14 (7.5)	6.83 (7.7)	6.72	6.63 (7.4)	8.09 (d, 7.0, 2H, H-2`,6`),7.49 (m, 3H, H-3`4`5`)
2.2	14.36	7.87	7.15 (7.2)	6.85 (8.1)	6.75	6.65 (7.3)	8.11 (t, *J* = 7.3 Hz, 1H, H-6`), 7.50 (bs, 1H, H-4`), 7.35 (m, 2H, H-3`, 5`)
2.3	13.91	7.80 (7.2)	7.33 – 7.22 (m, 2H, H-5, H-4`),	6.74 (8.1)	6.26	6.58 (7.3)	7.92 (d, *J* = 6.7 Hz, 1H, H-6`), 7.43 (d, *J* = 7.4 Hz, 1H, H-3`), 7.04 (t, *J* = 7.3 Hz, 1H, H-5`)
2.4	14.40	8.07 – 7.69 (m, 3H, H-3, H-3`,6`)	7.14 (6.6)	6.82 (8.0)	6.76	6.63 (7.3)	7.60 – 7.27 (m, 2H, H-3`, H-4`)
2.5	14.49	7.84 (m, 4H, H-3, H-3`5`6`), 7.17 (t, 1H, H-4`), 6.74 (m, 3H, H-4,5,6)					
2.6	13.54	8.05 (7.5)	7.37 (7.7)	6.75 (8.1)	6.29	6.59 (7.4)	8.24 (d, *J* = 6.9 Hz, 1H, H-6`), 7.05 (m, 3H, H-3`, 4`, 5`), 3.98 (s, 3H, OCH_3)
2.7	14.42	7.94 (7.5)	7.14 (7.2)	6.84 (8.1)	6.71	6.62 (7.2)	7.83 (m, 2H, H-2`,6`), 7.54 (dd, *J* = 13.8, 7.2 Hz, 1H, H-5`), 7.26 (t, *J* = 8.0 Hz, 1H, H-4`)
2.8	–	7.88 (7.8)	7.11 (7.6)	6.82 (8.2)	6.71	6.60 (7.4)	8.10 (s, 1H, H-2`), 8.06 (d, *J* = 7.5 Hz, 1H, H-6`), 7.57-7.43 (m, 2H, H-4`,5`)
2.9	14.15	7.75 (6.2)	7.08 (7.2)	6.79 (7.8)	6.26	6.59 (7.3)	8.24 (s, 1H, H-2`), 8.08 (d, *J* = 7.7 Hz, 1H, H-6`), 7.49 (d, *J* = 6.8 Hz, 1H, H-4`), 7.35 (t, *J* = 7.5 Hz, 1H, H-5`)
2.10	14.61/14.30	Overlaps on 3-Ar	7.13 (7.2)	6.83 (7.2)	–	6.61 (7.4)	8.41 – 8.30 (m, 2H, H-2`,6`), 7.70 (m, 3H, H-3, H-4`,5`)
2.11	–	7.83 (5.8)	7.08 (7.5)	6.94 (8.0)	–	6.59 (7.4)	7.64 (d, *J* = 7.6 Hz, 1H, H-6`), 7.59 (s, 1H, H-2`), 7.36 (t, *J* = 7.9 Hz, 1H, H-5`), 6.79 (d, *J* = 8.1 Hz, 1H, H-4`), 3.83 (s, 3H, OCH_3)
2.12	–	Overlaps on 3-Ar	7.25 (6.9)	7.06 (7.4)	6.22	6.88 (6.9)	8.03 (m, 3H, H-3, H-2`,6`), 7.33 (d, *J* = 7.2 Hz, 2H, H-3`,5`), 2.37 (s, 3H, CH_3)
2.13	14.36	7.83 (7.3)	7.15 (7.3)	6.85 (7.8)	–	6.64 (7.0)	8.13 (t, *J* = 6.7 Hz, 2H, H-2`,6`), 7.34 (t, *J* = 7.7 Hz, 2H, H-3`,5`)
2.14	–	8.11 (7.0)	7.57 (7.0)	7.16 (7.0)	6.84-6.63		8.30 (d, *J* = 7.1 Hz, 2H, H-2`6`), 7.85 (d, 2H, *J* = 7.1 Hz, 2H, H-3`5`)
2.15	–	7.62 (8.0)	7.15 (7.3)	6.85 (8.0)	–	6.63 (7.3)	7.85 (d, *J* = 8.0 Hz, 2H, H-2`,6`), 7.66 (d, *J* = 7.9 Hz, 2H, H-3`,5`)
2.16	14.60/14.23	Overlaps on 3-Ar	7.11 (7.6)	6.82 (7.5)	6.73	6.60 (7.4)	8.29 (d, *J* = 8.0 Hz,2H, H-2`6`), 7.74 (m, 3H, H-3, H-3`,5`)
2.17	–	8.04 (7.6)	7.29 (7.5)	7.13 (7.9)	–	6.96 (7.0)	8.10 (d, *J* = 8.4 Hz, 2H, H-2`,6`), 7.09 (d, *J* = 8.5 Hz, 2H, H-3`,5`), 3.83 (s, 3H, OCH_3)

Table 3

^{13}C NMR-spectra of [2-(3-aryl-1*H*-1,2,4-triazole-5-yl)phenyl]amine (**2.1**-**2.17**)

Compd.	^{13}C NMR, δ (ppm), *J* (Hz)								
	triaz. C-3	triaz. C-5	C-1	C-5	C-3	C-4	C-6	C-2	3-Ar
2.1	160.73	154.13	147.44	129.20	126.44	116.50	115.28	108.96	131.40 (C-1,3,4,5), 127.47 (C-2,6)
2.2	162.81	154.66	146.83	129.72	124.59	116.02	115.16	109.26	159.30 (C-2), 131.12 (C-5), 130.32 (C-4), 127.18 (C-6), 116.36 (C3)
2.3	161.74	156.78	147.02	131.47	126.83	116.55	115.81	109.90	132.48 (C-2), 130.64 (C-1, 3), 130.44 (C-6), 130.00 (C-4), 127.57 (C-5)
2.4	159.40	154.54	146.80	130.33	126.99	116.01	115.11	107.95	133.54 (C-3), 131.54 (C-1), 131.27 (C-5), 131.01 (C-6), 127.47 (C-4), 121.00 (C-2)
2.5	158.89	154.83	147.10	131.44	126.54	116.14	115.07	107.37	132.27 (C-5), 132.06 (C-2), 130.81 (C-4), 130.30 (C-1), 129.26 (C-6), 126.67 (C-3), 123.87 (q, *J* = 276.9 Hz, CF_3)
2.6	160.58	150.79	146.33	130.71	120.30	115.53	115.03	112.80	156.45 (C-2), 129.14 (C-6), 128.91 (C-4), 127.93 (C-5), 115.79 (C-1), 110.97 (C-3), 55.05 (OCH_3)
2.7	158.10	154.18	147.50	130.97	127.65	116.60	115.60	109.28	162.88 (C-3), 133.00 (C-5), 131.34 (C-1), 122.43 (C-6), 116.44 (C-4), 112.96 (C-2)
2.8	158.15	157.65	147.40	131.12	125.86	116.46	115.56	110.02	134.01 (C-3), 132.99 (C-1), 130.61 (C-5), 129.17 (C-2), 127.77 (C-4), 124.88 (C-6)
2.9	159.32	155.84	147.28	131.58	127.33	116.53	115.56	108.54	134.12 (C-1), 130.68 (C-5), 130.46 (C-2), 129.04 (C-4), 125.12 (C-6), 122.50 (C-3)
2.10	158.93	155.64	146.96	132.10	126.86	116.06	115.04	107.83	130.27 (C-1), 129.79 (C-5), 129.33 (C-6), 124.99 (C-4), 123.78 (C-3), 122.06 (C-2)
2.11	161.50	154.15	147.09	130.42	127.79	116.50	115.35	108.57	159.87 (C-3), 130.05 (C-1, 5), 118.81 (C-6), 115.69 (C-4), 111.58 (C-2), 55.43 (OCH_3)
2.12	157.36	152.59	142.28	130.12	127.57	118.37	113.39	110.64	139.31 (C-4), 130.12 (C-1), 129.32 (C-3,5), 126.03 (C-2,6), 20.91 (CH_3)
2.13	159.35	155.65	146.72	130.02	127.17	116.11	115.26	108.00	162.68 (C-4), 130.45 (C-1), 128.09 (C-2,6), 115.70 (C-3,5)
2.14	161.94	157.48	146.98	130.48	125.65	116.05	115.09	108.76	134.10 (C-1, 4), 128.81 (C-3,5), 126.41 (C-2,6)
2.15	162.65	158.86	146.74	130.87	124.77	116.14	115.23	110.90	131.49 (C-3,5), 131.14 (C-2,6), 129.88 (C-1), 122.00 (C-4)
2.16	160.38	155.44	146.81	130.33	126.74	116.08	114.96	107.78	137.89 (C-1), 128.52 (C-4), 127.62 (C-3,5), 125.29 (Ph C-2,6), 124.25 (CF_3)
2.17	158.56	157.08	141.31	130.60	119.67	114.86	114.73	110.02	161.05 (C-4), 128.26 (C-2,6), 121.24/120.53 (C-1), 55.74 (OCH_3)

by formation of $[M–N_2]^{+\bullet}$ fragmental ion; the second one by breaking N(1)–N(2) and C(3)–N(4) followed by formation of $[M–o\text{-}NH_2C_6H_4CN]^{+\bullet}$ ion (m/z 118). At the same time for compounds with an electron acceptor substituent (2.4, 2.13, 2.14, 2.16) only fragmentation by N(1)–N(2) and C(3)–N(4) bonds followed by formation of $[M–o\text{-}NH_2C_6H_4CN]^{+\bullet}$ ion (m/z 118) was typical. Moreover, all compounds studied formed $[M–o\text{-}NH_2C_6H_4\text{-}CHN]^{+\bullet}$ ion (m/z 119). As we consider, formation of alternative ions with m/z 119 and 118 may be explained by existence of 1,2,4-triazol in the gas phase as two tautomer forms (A and C).

The spectral data of 10.1-10.6 presented allowed to prove the structure of the given compounds, but for undeniable elucidation of the nature of the molecule formed, as well as for clarification of a tautomeric form in the crystal state the X-ray structural study for compound 2.15 was used.

All non-hydrogen atoms of molecule 2.15 with the exception of fluorine atoms lie in the plane within 0.02 Å (Fig. 1). Planar conformation is stabilized by the N4-H4Nb…N3 intramolecular hydrogen bond (H…N 2.05 Å N-H…N 126°) and the H14…N1 and H10…N3 attractive interactions (H…N are 2.59 Å and 2.60 Å, respectively, compared to the van der Waals radii sum [31] 2.67 Å); it may not be considered as hydrogen bonds due to too small bond angles (99° and 101°, respectively, Fig. 1). In the crystal phase molecules 2.15 form corrugated chains along the [0 1 0] crystallographic direction due to formation of N2-H…N4' (0.5-x, y-0.5, z) H…N 2.01 Å N-H…N 161°. The participation of the lone pair of the N4 atom in this hydrogen bond results in pyramidal configuration of the amino group (the sum of bond angles centered at the N4 atom is 335°). The neighbouring chains are bonded by the N4-H4Na…N1' (-x, 0.5+y, 0.5-z) intermolecular hydrogen bond (H…N 2.43 Å N-H…N 135°).

Thus, it was shown that 2-aryl-[1,2,4]triazolo[1,5-c]quinazolines under the action of nucleophilic agents

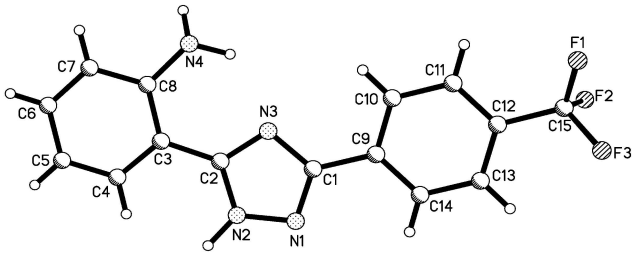


Fig. 1. The molecular structure of compound 2.15 according to X-ray diffraction data.

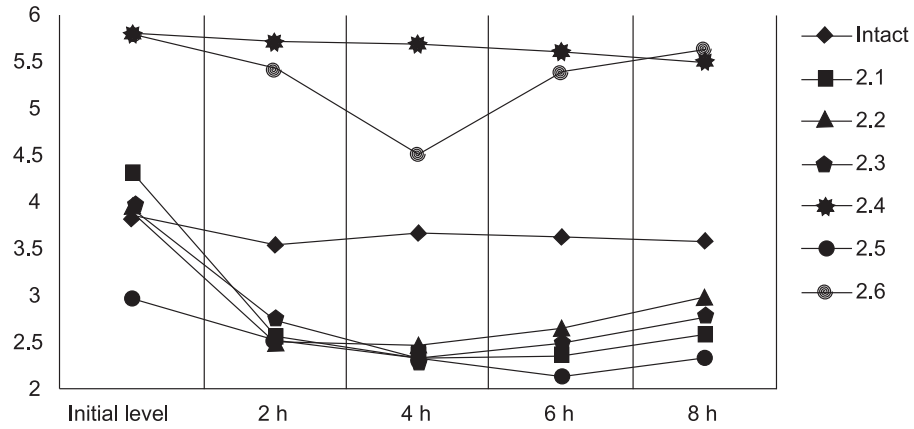


Fig. 2. The results of studying the hypoglycemic activity of [2-(3-phenyl-(2.1) 3-(2-R-phenyl)-(2.2-2.6)-1*H*-[1,2,4]triazolo-5-yl)phenyl]amines.

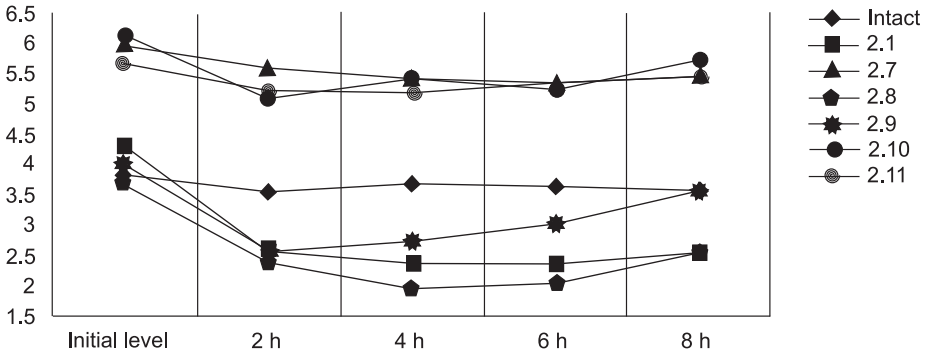


Fig. 3. The results of studying the hypoglycemic activity of [2-(3-phenyl- (2.1) 3-(3-R-phenyl)-(2.7-2.11)-1*H*-[1,2,4]triazolo-5-yl)phenyl]amines.

undergo hydrolytic cleavage followed by formation of [2-(3-aryl-1H-1,2,4-triazol-5-yl)phenyl]amines. The prototropic azole-azole tautomery has been also proven using physicochemical methods. We noted that the compounds synthesized exist as A-forms in crystals and as the equilibrium system of A and C-forms in dimethylsulfoxide solutions and the gas phase.

2.2. Hypoglycemic activity

The results of the preliminary hypoglycemic activity assay are presented in Fig. 2-4. Thus, [2-(3-phenyl-1*H*-[1,2,4]triazolo-5-yl)phenyl]amine (2.1) decreases the blood glucose level of normoglycemic rats within the whole experiment. The glucose level was also lowered by injection of compounds 2.2, 2.3 and 2.5 containing fluorine, chlorine and trifluormethyl substituents in position 2 of the 3-phenyl fragment (Fig. 2). At the same time compounds containing 2-bromphenyl (2.4) and 2-methoxyphenyl (2.6) moieties in position 3 of the triazole cycle increased the glucose level within the whole experiment.

Translocation of substituents in the phenyl moiety from *o*- (compounds 2.2-2.6) to *m*- (2.7-2.11) position significantly changes the action of compounds (Fig. 3). Thus, most effective glucose lowering agents were compounds 2.8 and 2.9 containing chlorine and bromine. At the same time compounds with 3-fluoro- (2.7), 3-trifluoromethyl (2.10) and 3-methoxyphenyl (2.11) increased the blood glucose level.

Further modification of the compounds synthesized, namely translocation of substituents of the phenyl moiety from position 3 (2.7-2.11) to position 4 (2.12-2.17), also led to ambiguous results. Experimental data have

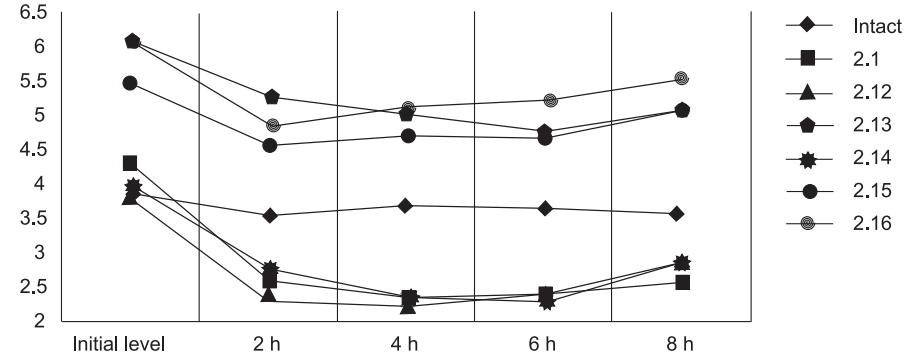


Fig. 4. The results of studying the hypoglycemic activity of [2-(3-phenyl-(2.1) 3-(4-R-phenyl)-(2.12-2.16)-1*H*-[1,2,4]triazolo-5-yl)phenyl]amines.

Table 4

Results of the specific pharmacologic action evaluation of the compounds synthesized (oral test for glucose tolerance)

Compd.	Initial glucose level	Glucose level in 15 min	% increase	Glucose level in 30 min	% increase	Glucose level in 60 min	% increase	Glucose level in 120 min	% increase
Control	5.9±0.1	7.7±0.3	30.5±4.4	8.7±0.2	47.3±2	9.4±0.1	58.6±4.7	6.8±0.2	15.7±2.2
Intact	5.6±0.3	7.5±0.3	35.6±6.2	7.8±0.4	39.9±7.9	6.8±0.3	23.3±7.7[a]	5.5±0.2	-0.03±6.4[a]
Metformin 200	5.5±0.2	6.8±0.1	22.9±2.2	7.5±0.1	37.0±2.9[a]	7.1±0.2	29.6±1.7[a]	6.7±0.3	22.2±1.8[ab]
Metformin 50	5.5±0.3	6.1±0.5	9.3±2.7[a]	7.4±0.5	33.0±1.5[a]	7.6±0.6	36.5±4.2[a]	5.9±0.4	5.9±3.0[a]
Gliclazide 50	5.7±0.1	7.7±0.1	34.5±4.8	7.9±0.1	37.3±3.0[a]	7.3±0.3	27.8±3.02[a]	6.2±0.1	7.7±3.1
2.1	5.3±0.1	7.1±0.1	34.8±1.8	8.1±0.1	52.6±2.3	7.1±0.2	34.9±3.3[a]	6.1±0.3	15.1±4.5
2.8	6.1±0.1	7.4±0.1	20.8±0.9	8.3±0.7	35.7±8.8	6.9±0.1	13.7±0.8[a]	6.7±0.2	9.3±2.2
2.12	5.6±0.5	8.1±0.4	46.4±8.4	8.7±0.6	55.9±4.6	9.3±0.7	66.4±5.3	7.7±0.5	38.6±5.7[a]
2.14	5.5±0.1	7.0±0.3	27.7±3.7	7.5±0.5	36.1±6.5	7.7±0.3	39.3±3.6[a]	5.8±0.2	5.4±1[a]

[a] significant differences (p≤0.05) compared to the control group of rats;
[b] significant differences (p≤0.05) compared to the intact group of rats.

shown that some compounds (2.12, 2.14) significantly decrease the level of glucose, but other 2.13, 2.15 and 2.16 have quite opposite type of action (Fig. 4).

Thus, the preliminary screening conducted showed that among the compounds synthesized the most active were 2.1, 2.8, 2.12, 2.14. It was found that anilines 2.8 and 2.12 in the phenyl moiety contained a chlorine atom. At the next stage of our investigation the hypoglycemic action of compounds 2.1, 2.8, 2.12, 2.14 was studied using the glucocorticoid-induced insulin resistance model. Resistance to insulin was formed by the injection of dexamethasone.

Evaluation of the specific hypoglycemic action (oral test for glucose tolerance) in conditions of glucocorticoid-induced insulin resistance showed that compounds 2.1, 2.8, 2.14 exhibited the hypoglycemic action, which was comparable to the activity of the reference-drugs "Metformin" (in the doses of 50 and 200 mg/kg) and Gliclazide (in the dose of 50 mg/kg, Tab. 4). At the same time compound 2.12 was inactive in the assay mentioned.

Further investigation of compounds 2.1, 2.8, 2.12, 2.14 in rats with glucocorticoid-induced insulin resistance using the rapid insulin test (Tab. 5) allowed to obtain results, which were concordant with the data of the previous experiments and showed that compounds 2.8 and 2.14 exhibited the hypoglycemic action at the

Table 5

Results of the specific pharmacologic action evaluation of the compounds synthesized (rapid insulin test)

Compounds	Initial glucose level	Glucose level in 30 min	% decrease of the glucose level
Control	5.7±0.2	4.3±0.1	-24.6±1.1[b]
Intact	5.7±0.1	2.3±0.1	-60.4±0.9[a]
Metformin 200	5.6±0.2	3.6±0.1	-34.6±3.7[ab]
Metformin 50	5.2±0.2	3.7±0.2	-29.8±3.3[b]
Gliclazide 50	5.4±0.2	3.5±0.2	-34.9±2.8[ab]
2.1	5.4±0.2	4.0±0.1	-25.7±3.6[b]
2.8	5.0±0.5	3.6±0.8	-45.4±1.3[ab]
2.12	5.0±0.3	3.3±0.2	-27.3±2.8[b]
2.14	5.4±0.4	3.2±0.1	-40.4±3.7[ab]

[a] – significant differences (p≤0.05) compared to the control group of rats; [b] – significant differences (p≤0.05) compared to the intact group of rats.

Table 6

Results of the specific pharmacologic action evaluation of the compounds synthesized (adrenaline test)

Compounds	Initial glucose level	Glucose level in 30 min	% increase of the glucose level	Glucose level in 30 min	% increase of the glucose level
Control	5.1±0.1	12.4±0.5	144.2±7.9[b]	16.7±0.2	228.9±6.1[b]
Intact	5.6±0.2	11.5±0.4	104.8±2.7[a]	13.7±0.6	144.3±5.7[b]
Metformin 200	6.7±0.2	9.6±0.2	42.2±2.8[ab]	10.9±0.1	63.3±4.9[ab]
Metformin 50	5.5±0.1	6.7±0.2	21.2±3.1[ab]	10.6±0.2	92.2±4.9[ab]
Gliclazide 50	6.0±0.1	7.7±0.2	28.4±5.9[ab]	10.1±0.2	66.4±4.4[ab]
2.1	5.1±0.1	9.8±0.3	93.4±1.8[ab]	8.8±0.7	73.5±10.3[ab]
2.8	6.4±0.1	7.9±0.3	23.5±2.9[ab]	9.2±0.4	43.8±2.8[ab]
2.12	7.1±0.4	13.8±2.4	94.5±24.4	17.5±3.3	147.3±33.8
2.14	6.3±0.3	9.4±0.5	50.5±12.9[ab]	12.2±0.7	93.9±5.2[ab]

[a] – significant differences (p≤0.05) compared to the control group of rats; [b] – significant differences (p≤0.05) compared to the intact group of rats.

level similar to the reference drugs. Thus, the hypoglycemic activity of [2-(3-chloro-1*H*-[1,2,4]-triazole-5-yl) phenyl]aniline (2.8) exceeded the action of Gliclazide by 10.5-15.6%.

We noted that [2-(3-chloro-1*H*-[1,2,4]-triazole-5-yl) phenyl]amine (2.8) exhibited a high hypoglycemic activity in rats with glucocorticoid-induced insulin resistance using the adrenaline test. Thus, compound 2.8 exceeded the action of the reference drugs by 19.5-48.4% (Tab. 6).

CONCLUSIONS

According to the data of the specific pharmacologic action evaluation of the compounds synthesized (oral test for glucose tolerance, rapid insulin test and adrenaline test) the hypoglycemic action of 2-(3-aryl-H-1,2,4-triazol-5-yl)phenyl]amines (2.1, 2.14) in the dose of 10 mg/kg is not inferior to the action of the reference drugs Metformin (in the doses of 50 and 200 mg/kg) and Gliclazide (in the dose of 50 mg/kg), moreover compound 2.8 exceeds the activity of the drugs mentioned above.

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ГІДРОЛІТИЧНЕ РОЗЩЕПЛЕННЯ ПІРИМІДИНОВОГО ЦИКЛУ В 2-АРИЛ-[1,2,4]ТРИАЗОЛО[1,5-с]ХІНАЗОЛІНАХ: ФІЗИКО-ХІМІЧНІ ВЛАСТИВОСТІ ТА ГІПОГЛІКЕМІЧНА АКТИВНІСТЬ СИНТЕЗОВАНИХ СПОЛУК

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Ключові слова: *2-арил-[1,2,4]триазоло[1,5-с]хіназоліни; гідролітичне розщеплення; гіпоглікемічна активність*

Показано, що 2-арил-[1,2,4]триазоло[1,5-с]хіназоліни під дією нуклеофільних реагентів (гідразин гідрат, гідроксид або мелилат натрію, кислота хлористоводнева) піддаються гідролітичному розщепленню з утворенням 2-(3-арил-1Н-1,2,4-триазол-5-іл)феніл]амінів. Запропоновані оптимальні умови синтезу: хлористоводнева кислота, спирто-водне середовище, температура 90-95°С, тривалість 60 хвилин. Встановлено, що за запропонованих умов синтезу не спостерігається суттєвого впливу замісника положення 2 триазолохіназолінового циклу на тривалість реакції та виходи кінцевих продуктів. Індивідуальність синтезованих сполук підтверджена хроматомас-спектрометрією, будова – елементним аналізом, ^{1}Н, ^{13}С ЯМР-, мас-спектрами та рентгеноструктурним аналізом. За допомогою фізико-хімічних методів обгрунтована азол-азольна (прототропна) таутомерія синтезованих сполук. Показано, що в розчинах ДМСО та газовій фазі 2-(3-арил-1Н-1,2,4-триазол-5-іл)феніл]аміни переважно існують у вигляді А та С-форм, тоді як у кристалічній решітці існують у вигляді А-форми. Встановлено, що 2-(3-арил-1Н-1,2,4-триазол-5-іл)феніл]аміни (2.1, 2.8, 2.14) в дозі 10 мг/кг при визначенні специфічної фармакологічної активності, а саме оральному тесті толерантності до глюкози (ОТТГ)), короткому інсуліновому та адреналіновому тесті не поступаються за гіпоглікемічною активністю референс-препаратам «Метформіну» (доза 50 та 500 мг/кг) та «Гліклазиду» (доза 50 мг/кг).

ГИДРОЛИТИЧЕСКОЕ РАСЩЕПЛЕНИЕ ПИРИМИДИНОВОГО ЦИКЛА 2-АРИЛ-[1,2,4]ТРИАЗОЛО[1,5-с]ХИНАЗОЛИНОВ: ФИЗИКО-ХИМИЧЕСКИЕ СВОЙСТВА И ГИПОГЛИКЕМИЧЕСКАЯ АКТИВНОСТЬ СИНТЕЗИРОВАННЫХ СОЕДИНЕНИЙ

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Ключевые слова: *2-арил-[1,2,4]триазоло[1,5-с]хиназолины; гидролитическое расщепление; гипогликемическая активность*

В представленной работе показано, что 2-арил-[1,2,4]триазоло[1,5-с]хиназолины под действием нуклеофильных реагентов (гидразин гидрат, гидроксид натрия или метилат натрия, кислота хлористоводородная) подвергаются гидролитическому расщеплению с образованием 2-(3-арил-1Н-1,2,4-триазол-5-ил)фенил]аминов. Предложены оптимальные условия синтеза: хлористоводородная кислота, спирто-водная среда, температура 90-95°С, продолжительность 60 минут. Установлено, что в предложенных условиях синтеза не наблюдается значительного влияния заместителя положения 2 триазинохиназолинового цикла на продолжительность реакции и выходы конечных продуктов. Индивидуальность синтезированных соединений подтверждена хроматомас-спетрометрией, строение – элементным анализом, ^{1}Н, ^{13}С ЯМР-, масс-спектрами и рентгеноструктурным анализом. С использованием физико-химических методов обоснована азол-азольная (прототропная) таутомерия синтезированных соединений. Показано, что в растворах ДМСО и газовой фазе 2-(3-арил-1Н-1,2,4-триазол-5-ил)фенил]амины главным образом существуют в виде А и С-форм, тогда как в кристаллической решетке в виде А-формы. Установлено, что 2-(3-арил-1Н-1,2,4-триазол-5-ил)фенил]амины (2.1, 2.8, 2.14) в дозе 10 мг/кг при оценке гипогликемической активности в оральном тесте толерантности к глюкозе (ОТТГ), коротком инсулиновом и адреналиновом тесте не уступают по гипогликемической активности референс-препаратам «Метформину» (доза 50 и 500 мг/кг) и «Гликлазиду» (доза 50 мг/кг).

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

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DEVELOPMENT OF THE SPECTROPHOTOMETRIC METHOD FOR QUANTITATIVE DETERMINATION OF METRONIDAZOLE IN CAPSULES

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Key words: metronidazole; capsules; spectroscopy, assay

Pathological conditions of the digestive system are quite common nowadays and rank first among other human diseases. Increasingly, both newly-diagnosed and recurrent cases of stomach ulcers, as well as duodenal ulcers are recorded. Among solid drug dosage forms capsules are considered to be the most rational since the drug substance exhibits a low degree of dispersion, which leads to better absorption, resists additional negative impact in the production process, as well as possesses a lower damaging action on the mucous membranes of the digestive tract, etc. Thereby capsules are more and more often recommended for use by preference. Therefore, developing a feasible method of quantitative determination of metronidazole in capsules – the method of spectrophotometry in the ultraviolet region of the spectrum has become particularly urgent since high performance liquid chromatography recommended by the BP and USP is not used widely in Ukraine. The object of research was metronidazole capsules. The research has found that the excipients have little effect on the absorption spectrum of metronidazole obtained by extraction with 0.1 M hydrochloric acid solution from the content of capsules. The test solution has been proven to be complied with the Beer-Lambert-Bouguer law within the concentration range of $0.5 \cdot 10^{-3}$% – $3.0 \cdot 10^{-3}$%. The certification of the method developed on the model mixtures and a production sample has confirmed its correctness. The average results uncertainty ε in the former case was 0.11%, whereas in the latter it constituted 0.70%.

Pathological conditions of the digestive system are quite common nowadays and rank first among other human diseases. Thus, the most common of these is ulcer of the stomach and duodenum [5, 7, 10, 12]. As recommended by the Maastricht consensus two lines of therapy containing metronidazole are used for treatment of this disease [14, 16-18]. Among solid drug dosage forms capsules are considered to be the most rational since they have several significant advantages, including high bioavailability, increased therapeutic effect, reduction of the damaging action on the mucous membranes of the gastrointestinal tract, prolongation of the action to reduce a negative impact on the drug substance in the production process, etc. [8, 16].

First of all, the necessary condition for safe use of medicines is the existence of quality control methods available in each particular region. The State Pharmacopoeia of Ukraine (SPhU) recommends to determine the quantitative content of metronidazole capsules by using the liquid chromatography method [3]. However, it should be noted that for Ukraine this method is not widely available because of the high cost of the analysis and impossibility of providing the drug quality control laboratories with chromatographs on a large scale [9].

The aim of our paper was to develop more available method for determining the quantitative content of metronidazole in the form of capsules by spectrophotometry in the ultraviolet region of the spectrum.

Materials and Methods

The substance of metronidazole (manufacturer: Luotian Hongyuan Biochemical Co., LTD, China, batch 08111803) and "TRIKACIDE" capsules containing 500 mg of metronidazole (manufacturer: Pharmascience Inc., 6111, Royalmaunt Avenue, Montreal, Quebec, Canada, batch 6452653) meeting the requirements of the SPhU were chosen as the objects of study.

Such analytical equipment as an "Evolution 60S" Spectrophotometer (USA), AB 204 S/A METTLER TOLEDO analytical balance, as well as glassware for measuring, class A (first class), and reagents meeting the requirements of the SPhU was used in the study.

The method of study was absorption spectrophotometry in the ultraviolet and visible regions by the method of standard.

All solutions were prepared in accordance with the SPhU requirements [2, 3, 6, 11, 13, 18, 19].

Construction of the Calibration Curve. Place 0.1000 (accurate weight) of metronidazole standard sample (SS) in a 100.0 ml volumetric flask, then dissolve in 0.1 M hydrochloric acid solution, dilute with the same solvent to the volume and mix thoroughly. Take the aliquot of 0.5 ml; 1.0 ml; 1.5 ml; 2.0 ml; 2.5 ml; 3.0 ml with a pipette in a 100.0 ml volumetric flask, dilute to the volume with 0.1 M hydrochloric acid solution and mix.

Assay of Metronidazole in Capsules. Place the accurate weight of the content of 20 capsules equivalent

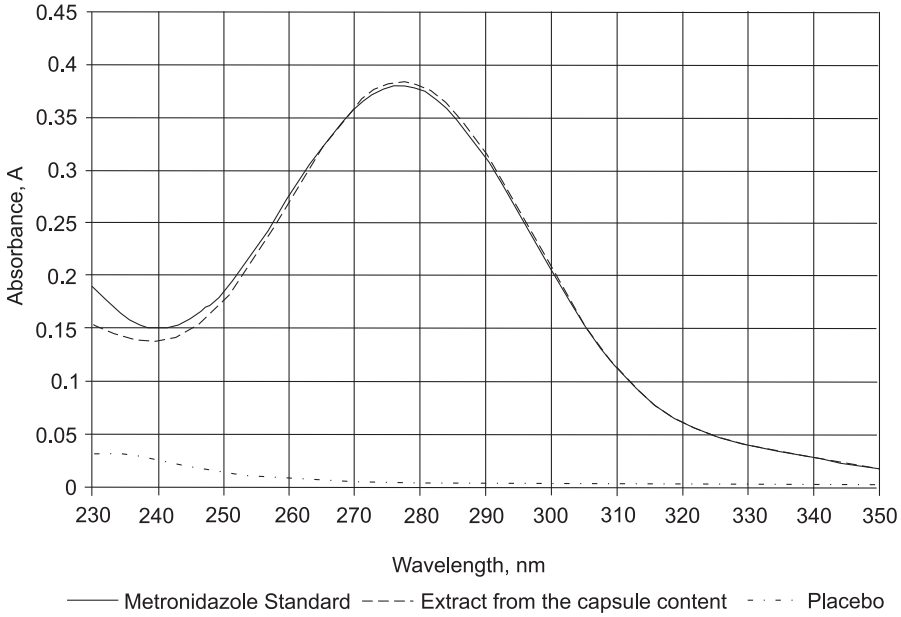

Fig. 1. The effect of excipients on the UV absorption spectrum of the extract from the metronidazole capsules content.

to 0.1000 g of metronidazole (approximately 0.1209 g) in a 100.0 ml volumetric flask, add 50 ml of 0.1 M hydrochloric acid solution, shake for 15 min, dilute with the same solvent to the volume. Filter the solution obtained, reject the first and the last portions of the filtrate. Take the aliquot of 1.0 ml of the solution obtained, place in a 100.0 ml volumetric flask and dilute with the same solvent to the volume. Measure the absorbance on a spectrophotometer at the wavelength of 277 nm in a cell with the layer thickness of 10 mm. In parallel, perform the measurement of the metronidazole standard sample solution.

Preparation of Metronidazole Standard Sample Solution. Place 0.1000 g of metronidazole standard sample in a 100.0 ml volumetric flask, dissolve in 0.1 M hydrochloric acid solution, and dilute with the same solvent to the volume. Take the aliquot of 1.0 ml of the solution obtained and place in a 100.0 ml volumetric flask and dilute with the same solvent to the volume.

Preparation of Placebo Solution. Place 90.50 mg of microcrystalline cellulose, 7.00 mg of silicon dioxide and 7.00 mg of magnesium stearate in a 100.0 ml volumetric flask, and dissolve in 0.1 M hydrochloric acid solution, mix thoroughly, dilute with the same solvent to the volume. Filter the solution obtained, reject the first and the last portions of the filtrate. Take the aliquot of 1.0 ml of the solution obtained in a 100.0 ml volumetric flask and dilute with the same solvent to the volume.

Compensation solution. 0.1 M solution of hydrochloric acid.

Calculation formula:

$$X, mg = \frac{A \cdot m_{st} \cdot 100.0 \cdot 100.0 \cdot 1.0 \cdot m_{a.m.caps.}}{A_{st} \cdot m_{caps.} \cdot 1.0 \cdot 100.0 \cdot 100.0},$$

where: A – is absorbance of *Test solution*; A_{st} – is absorbance of *Standard solution*; $m_{caps.}$ – is the weight amount of the drug studied; m_{st} – is the weight amount of the standard sample; $m_{a.m.caps.}$ – is the average mass of the content of 20 capsules in the batch under study.

Results and Discussion

As a solvent 0.1 M hydrochloric acid solution was chosen, which the world's leading Pharmacopoeias [3, 11, 15, 20] recommend to use for the assay of metronidazole in the substance and the dosage forms.

First of all, the influence of excipients on the absorption spectrum of metronidazole was determined. In order to do that, the absorption spectra of the metronidazole standard solution, the extract from the content of capsules and placebo solution were compared. The UV absorption spectrum of the extract of the content of metronidazole capsules almost coincides with the UV-spectrum of the metronidazole standard sample solution. The influence of optical absorption of the placebo solution on absorption of the extract solution from the capsules content is very insignificant at the analytical wavelength of 277 nm in the medium of 0.1 M hydrochloric acid solution (Fig. 1).

It has been found that the graph of dependence of the absorbance of metronidazole solution on its concentration is linear at the wavelength of $\lambda = 277$ nm (Fig. 2). The compliance of the test solutions with the Beer-Lambert-Bouguer law is observed within the concentrations from $0.5 \cdot 10^{-3}$% to 3.0×10^{-3}%. The average value of the specific absorbance in the experiment was 363.

The method for quantitative determination of metronidazole in capsules proposed was assessed on model mixtures. The results are presented in Tables 1 and 2. The uncertainty of the average result $\varepsilon = 0.11$% meeting the requirements of the SPhU.

The results of quantitative determination of metronidazole in capsules on the production sample of the drug and metrological characteristics obtained from the statistical processing of the measurement results, are presented in Tables 3 and 4 [1-3, 4, 6]. In this case, the uncertainty of the average result ε meets the SPhU and is 0.70%.

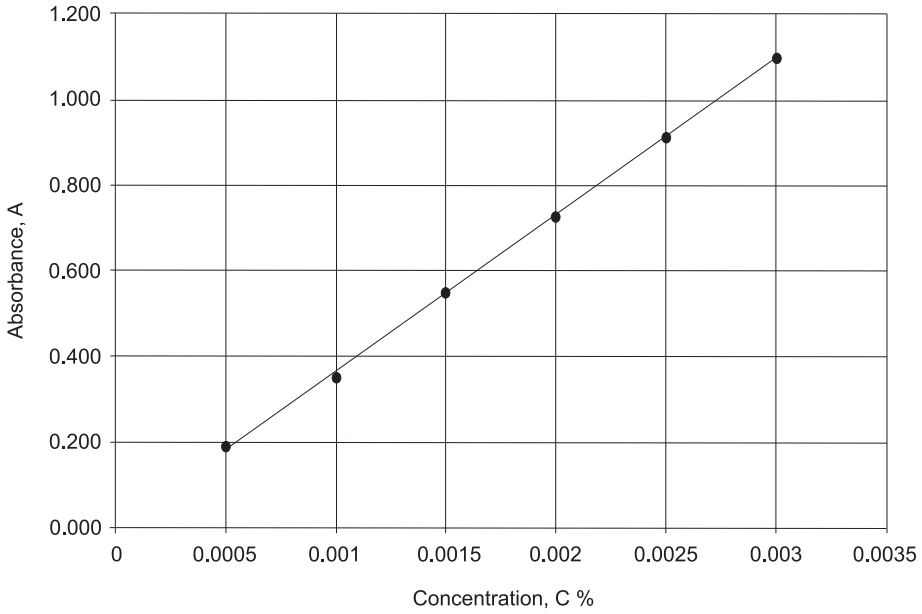

Fig. 2. The graph of dependence of absorbance on the concentration of metronidazole solution in 0.1 M hydrochloric acid solution.

Table 1

The results of quantitative determination of metronidazole in model mixtures

Metronidazole taken, g	The weight amount of the metronidazole standard sample, g	Absorbance of metronidazole	Absorbance of the metronidazole standard sample	Found, g
0.1000	0.1000	0.383	0.383	0.1000
		0.384	0.383	0.1003
		0.384	0.383	0.1003
		0.389	0.388	0.1001
		0.390	0.388	0.1004
		0.389	0.388	0.1001

Table 2

Metrological characteristics of the average results of quantitative determination of metronidazole in capsules. Testing on model mixtures

v	$\bar{x}$	S^2	S	$S_{\bar{x}}$	Δ_x	$\Delta_{\bar{x}}$	$\bar{\varepsilon}$, %
5	0.1002	0.0000000184	0.0001356	0.00005535	0.0002732	0.0001115	0.11

Table 3

The results of quantitative determination of metronidazole in capsules on the production sample

The metronidazole capsules content taken, g	The weight amount of the metronidazole standard sample, g	Absorbance of metronidazole	Absorbance of the metronidazole standard sample	Found, g ($m_{a.m.}$ = 0.6045)
0.1212	0.1000	0.387	0.382	0.5032
0.1205		0.385	0.382	0.5043
0.1216		0.381	0.382	0.4959
0.1210		0.378	0.382	0.4953
0.1213		0.378	0.382	0.4945
0.1211		0.381	0.382	0.4983

Table 4

Metrological characteristics of the average results of quantitative determination of metronidazole in capsules. Testing on the production sample

v	$\bar{x}$	S^2	S	$S_{\bar{x}}$	Δ_x	$\Delta_{\bar{x}}$	$\bar{\varepsilon}$, %
5	0.4986	0.00001787	0.004227	0.001726	0.00852	0.003477	0.70

Thus, these results confirm the possibility of quantitative determination of metronidazole in capsules by the method proposed.

CONCLUSIONS

1. The characteristics of metronidazole solution meeting the requirements of the method of spectrophotometry have been confirmed.

2. The insignificant effect of the excipients on absorption of metronidazole in capsules has been found.

3. The method for quantitative determination of metronidazole in capsules by spectrophotometry in the ultraviolet and visible regions of the spectrum has been developed for the first time.

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РОЗРОБКА СПЕКТРОФОТОМЕТРИЧНОЇ МЕТОДИКИ КІЛЬКІСНОГО ВИЗНАЧЕННЯ МЕТРОНІДАЗОЛУ В КАПСУЛАХ

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Ключові слова: *метронідазол; капсули; спектрофотометрія; кількісне визначення*

Патології органів травлення є досить поширеними на сьогодні та посідають перші місця серед інших захворювань людини. Все частіше фіксуються випадки як вперше виявлених виразкових дефектів шлунка та дванадцятипалої кишки, так і їх рецидиви. Серед твердих лікарських форм капсули вважають найбільш раціональними, оскільки лікарська речовина має малу дисперсність, що сприяє кращому всмоктуванню, не піддається додатковому негативному впливу в процесі виробництва, чинить меншу пошкоджувальну дію на слизові оболонки шлунково-кишкового тракту тощо. За рахунок цього все частіше рекомендують застосовувати саме капсули. У зв'язку з цим актуальним стало питання розробки доступного методу кількісного визначення метронідазолу в капсулах – методу спектрофотометрії в

ультрафіолетовій області спектра, оскільки високоефективна рідинна хроматографія, рекомендована BP та USP, не набула широкого застосування в Україні. Об'єктом дослідження було обрано капсули метронідазолу. В ході дослідження встановлено, що допоміжні речовини чинять незначний вплив на спектр поглинання розчину метронідазолу, отриманого шляхом екстракції зі вмісту капсул 0,1 М розчином кислоти хлористоводневої. Доведено підпорядкування досліджуваного розчину закону Бугера-Ламберта-Бера в межах концентрацій $0,5 \cdot 10^{-3}$% – $3,0 \cdot 10^{-3}$%. Атестація розробленої методики на модельних сумішах та серійному зразку препарату підтвердила її коректність. Невизначеність середнього результату ε в першому випадку становила 0,11%, а у другому – 0,70%.

РАЗРАБОТКА СПЕКТРОФОТОМЕТРИЧЕСКОЙ МЕТОДИКИ КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ МЕТРОНИДАЗОЛА В КАПСУЛАХ

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Ключевые слова: *метронидазол; капсулы; спектрофотометрия; количественное определение*

Патологии органов пищеварения являются достаточно распространенными на сегодняшний день и занимают первые места среди других заболеваний человека. Все чаще фиксируются случаи как впервые появившихся язвенных дефектов желудка и двенадцатиперстной кишки, так и их рецидивы. Среди твердых лекарственных форм капсулы считаются наиболее рациональными, поскольку лекарственное вещество имеет маленькую дисперсность, что способствует лучшему всасыванию, не подвергается дополнительному негативному влиянию в процессе производства, имеет более низкое повреждающее действие на слизистые оболочки желудочно-кишечного тракта и т. д. В связи с этим все чаще рекомендуют использовать именно данную форму. Поэтому актуальным является вопрос разработки доступного метода количественного определения метронидазола в капсулах – метода спектрофотометрии в ультрафиолетовой области спектра, поскольку высокоэффективная жидкостная хроматография, рекомендованная BP и USP, не имеет широкого применения в Украине. Объектом исследования стали капсулы метронидазола. В ходе исследования установлено, что вспомогательные вещества незначительно влияют на спектр поглощения раствора метронидазола, полученного путем экстракции из содержимого капсул 0,1 М раствором кислоты хлористоводородной. Доказано подчинение исследуемого раствора закону Бугера-Ламберта-Бера в границах концентраций $0,5 \cdot 10^{-3}$% – $3,0 \cdot 10^{-3}$%. Аттестация разработанной методики на модельных смесях и серийном образце препарата подтвердили ее корректность. Неопределенность среднего результата ε в первом случае составила 0,11%, а во втором – 0,70%.

ТЕХНОЛОГІЯ ЛІКАРСЬКИХ ПРЕПАРАТІВ

Recommended by Doctor of Pharmacy, professor N.P.Polovko

UDC 615.453.6:615.014.21

THE STUDY OF STABILITY OF THE COMBINED ANTIHYPERTENSIVE TABLETS DURING STORAGE

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Key words: combined tablets; amlodipine; indapamide; lisinopril; shelf life; stability

Creation of drugs for treating hypertension, namely the combined tablets based on antihypertensive agents of different pharmacological groups, is very important for domestic pharmaceutical science and practice. According to the results of the previous studies the composition and technology of the combined tablets "Amlopamide" for treating hypertension have been developed. As active ingredients these tablets contain amlodipine besylate, lisinopril dehydrate and indapamide. The excipients are microcrystalline cellulose, lactose monohydrate, potato starch and calcium stearate. The aim of this work was to study the physical and chemical, pharmaceutical and technological parameters, as well as microbiological purity of the combined tablets during storage. The modern methods of testing are used to determine stability of "Amlopamide" tablets obtained by direct compression in accordance with the State Pharmacopoeia of Ukraine. To determine the shelf life of the combined antihypertensive tablets "Amlopamide", as well as to study their stability, different batches of the drug were kept in different containers at a temperature of (20±5)°C. The control of the drug stability was performed according to all parameters: appearance, the average tablet weight, disintegration time, dissolution, uniformity of dosage units, identification, the assay content of active substances and microbiological purity. The results of the experimental studies have shown that during storage for 27 months at a temperature of (20±5)°C the samples of the combined tablets "Amlopamide" in all containers used by all parameters meet the requirements of normative documents and State Pharmacopoeia of Ukraine (SPhU). It has been determined that the shelf life of the tablets is 2 years while storing at room temperature.

Despite the obvious progress in development of drug therapy for treating hypertension this pathology is the most common cardiovascular risk factor in developed countries, including in Ukraine. One of the key links in achieving the target level of blood pressure (BP) in patients with hypertension is creation of fixed drug combinations, which considerably facilitate the process of achieving target BP, increase the activity of each drug compared to monotherapy, reduce the probability of side effects, increase compliance of patients to treatment and simultaneously reduce the cost of it [6, 9, 10]. According to the results of the previous studies together with employees of "CPP "Red Star" PJSC (Kharkov) the composition and technology of the combined three-component tablets under the conditional name "Amlopamide" for treating hypertension have been developed. As active ingredients these tablets contain amlodipine besylate (calcium channel blocker), lisinopril dehydrate (ACE inhibitor) and indapamide (thiazid-like diuretic). The excipients are microcrystalline cellulose, lactose monohydrate, potato starch and calcium stearate [4]. In the abovementioned context, creation of domestic combined drugs for treating hypertension is rather relevant for domestic pharmaceutical science and practice.

The quality control carried out in the technological processes of production of drugs guarantees the efficiency and safety of their use. At the present stage of development of domestic pharmaceutical industry and with introduction of the rules of good manufacturing practice (GMP) in the pharmaceutical enterprises of Ukraine the approaches to the quality control of drugs change. Standardization of the medicines developed, as well as determination of stability, conditions and the shelf life are important stages of their introduction into production [1, 5].

The aim of this work was to study the physical and chemical, pharmaceutical and technological parameters, as well as microbiological purity of the combined tablets during storage.

Experimental Part

To determine the stability of "Amlopamide" tablets obtained by direct compression the methods of physical and chemical (high performance liquid chromatography), pharmaceutical and technological, biological

studies were used in accordance with the State Pharmacopoeia of Ukraine (SPhU) by standard practice [1]. The quality control was performed according to the following parameters: appearance, identification, the average tablet weight, the loss on drying, friability, microbial purity, etc.

For identification and quantitative determination of active substances of the combined tablets "Amlopamide" the method of high performance liquid chromatography (HPLC) was used. This method allows determining compounds in very low concentrations. Determination of active ingredients – amlodipine besylate, lisinopril dehydrate and indapamide in the composition of drugs are mainly carried out by chromatographic and spectrophotometric methods [7, 8]. The presence of three active substances in the drug studied has determined the need for development of identification and quantitative determination methods, which allow to identify all active ingredients in tablets. Chromatographic analysis was performed on an Agilent 1100 liquid chromatograph with an UV-detector. To determine lisinopril in "Amlopamide" tablets the buffer solution with pH 7.0: acetonitrile *R* – water of chromatographic grade *R* (20:15:65) was used as a mobile phase; to determine amlodipine and indapamide the buffer solution with pH 7.0: acetonitrile *R* – water of chromatographic grade *R* (20:28:52) was used [2].

To provide the uniformity of dosage units (UDU) the content of active substances in a dosage unit in the batch must be within the narrow limits of the label claim. The UDU was studied by the method of direct determination according to the method of the SPhU for the presence of the labeled amount of active substances in the composition of the combined drugs [1]. Statistical processing of the results obtained was performed according to the SPhU (n = 5).

Results and Discussion

To determine the shelf life of the combined antihypertensive tablets "Amlopamide", as well as to study their stability, different batches of the drug were kept in different containers at room temperature (20±5)°C. The following types of containers were used: polymer jars for packaging of drugs with the first opening (TU U 00481318.001-98); blisters based on PVC film (GOST 25250-88) and printed lacquered aluminium foil (TU 48-21-270-88); amber glass jars of BDS-10-27.5-OC-1 type (TU 64-2-239-79) with stretched lids of type 1,2 (OST 64-2-87-81). The control of the drug stability was performed according to all parameters: appearance, the average tablet weight, disintegration time, dissolution, uniformity of dosage units, identification, the assay content of active substances and microbiological purity.

By appearance "Amlopamide" tablets are round, biconvex tablets of a white colour to white colour with a yellow tint. The observations showed the absence of such phenomena as stratification tablets, split edges, change of the tablet surface in colour. Determination of average tablet weight of the samples was conducted according to the requirements of the SPhU (of 20 tablets. Deviations in determining the average weight were not more than ±7.5%. It corresponds to the existing requirements.

The experimental results of studying the quality indicators of "Amlopamide" tablets developed are given in Table.

The results given in Table have shown that the combined tablets "Amlopamide" developed during storage at the temperature of (20±5)ºC studied in all containers give positive results by all parameters. It should be noted that at room temperature the tablets tend to increase in disintegration time. It is related to the fact that in the course of time the frame of the coat is thickened, and the penetration time of water through the capillaries of the micropores of the porous body increases, but during storage for 2 years and 3 months this parameter corresponds to the requirements of the SPhU.

Identification of active substances of the tablets was carried out using HPLC. The studies conducted have shown that on the chromatogram peaks and the retention times of the test solution were the same as peaks and retention times of standard solutions. While studying the quantitative content of the active substances of the combined tablets "Amlopamide", namely lisinopril dehydrate (calculated with reference to lisinopril), amlodipine besylate (calculated with reference to amlodipine) and indapamide (calculated with reference to 100% substance), varies within the permissible limits (±10%) during the whole period of storage [2].

The "Dissolution test" is the most important parameter in the study of kinetics of the active substances release from tablets. To study the release of active substances from "Amlopamide" tablets dissolution test for solid dosage forms was applied using a device with the blade [1]. Quantitative determination of active ingredients every 10 min for 60 min was performed using HPLC, water *R* was used as the dissolution medium. The studies have shown that release of lisinopril dehydrate (calculated with reference to lisinopril), amlodipine besylate (calculated with reference to amlodipine) and indapamide (calculated with reference to 100% substance) regardless of the shelf-life occurs within 80-95% for 45 min, and it meets the requirements of the SPhU [1].

The experimental data obtained on studying UDU of "Amlopamide" tablets indicate that by the quantitative content of active substances this dosage form stand the test for "Uniformity of dosage units" in accordance with the SPhU.

The microbiological purity test has shown that there are no bacteria of *Enterobacteriaceae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* families in the tablets. The viable aerobic microbial count of bacteria and fungi complies with the requirements of the SPhU for drugs for internal use [1, 3].

The results of the experimental studies have shown that during storage for 27 months at a temperature of (20±5)ºC the samples of the combined tablets "Amlopamide" in all containers used by organoleptic, pharmaceutical and technological parameters, as well as microbiological purity meet the requirements of normative documents and SPhU.

Table

The results of studying the stability of "Amlopamide" tablets during storage at the temperature of (20±5)°C

The name of parameters by QCM	The norm by QCM	Shelf life, months								
		Polymer jars			Blisters			Amber glass jars of BDS type		
		12	24	27	12	24	27	12	24	27
Appearance	White, round, biconvex tablets									
Identification Lisinopril Amlodipine Indapamide	On the chromatogram of the test solution obtained under the conditions of quantitative determination the retention times of peaks for lisinopril, amlodipine and indapamide are the same as the retention times of peaks for lisinopril, amlodipine and indapamide on the chromatogram of the standard solution with the accuracy of ±3%									
Average tablet weight	from 0.133 to 0.147 g	0.1385± 0.0266	0.1405± 0.0040	0.1401± 0.0132	0.1403± 0.0093	0.1395± 0.0095	0.1400± 0.0090	0.1403± 0.0092	0.1390± 0.0181	0.1393± 0.0193
Disintegration	not more than 15 min	3.2±0.3	4.5±0.2	5.1±0.3	4.0±0.4	4.3±0.2	5.0±0.4	3.9±0.3	4.5±0.2	5.3±0.2
Uniformity of dosage units	The acceptance value for the first 10 units should be not more than 15	Satisfied	Satisfied	Satisfied	Satisfied	Satisfied	Satisfied	Satisfied	Satisfied	Satisfied
Dissolution	for 45 min at least 75%	93.2±1.5	89.7±2.2	85.6±2.7	93.7±1.4	90.9±1.0	86.8±1.5	91.9±0.5	85.8±0.8	84.6±1.3
Microbiological purity	bacteria not more than 1000 fungi not more than 100	< 1000 < 100	< 1000 < 100	< 1000 < 100	< 1000 < 100	< 1000 < 100	< 1000 < 100	< 1000 < 100	< 1000 < 100	< 1000 < 100
The quantitative content: Lisinopril	in a tablet from 0.0045 to 0.0055 g	0.0050± 0.0001	0.0049± 0.0002	0.0051± 0.0001	0.0048± 0.0002	0.0049± 0.0001	0.0050± 0.0001	0.0048± 0.0002	0.0051± 0.0001	0.0050± 0.0001
Amlodipine	from 0.0045 to 0.0055 g	0.0048± 0.0002	0.0050± 0.0001	0.0049± 0.0002	0.0050± 0.0001	0.0049± 0.0001	0.0050± 0.0001	0.0049± 0.0001	0.0050± 0.0001	0.0051± 0.0001
Indapamide	from 0.00225 to 0.00275 g	0.00249± 0.00001	0.00252± 0.00001	0.00248± 0.00001	0.00251± 0.00001	0.00260± 0.00001	0.00258± 0.00001	0.00251± 0.00001	0.00262± 0.00001	0.00250± 0.00001

Note: n = 5. P = 95%.

CONCLUSIONS

The influence of storage conditions and types of containers on the quality of the combined antihypertensive tablets "Amlopamide" has been studied. The stability of organoleptic, pharmaceutical and technological, as well as microbiological parameters of tablets during storage for 27 months in amber glass jars, polymer jars and blisters at room temperature (20±5)ºC has been experimentally proven. It has been determined that the shelf life of the tablets is 2 years while storing at room temperature.

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ВИВЧЕННЯ СТАБІЛЬНОСТІ КОМБІНОВАНИХ АНТИГІПЕРТЕНЗИВНИХ ТАБЛЕТОК У ПРОЦЕСІ ЗБЕРІГАННЯ

О.П.Стрілець

Ключові слова: *комбіновані таблетки; амлодипін; індапамід; лізиноприл; термін придатності; стабільність*

Створення лікарських засобів для лікування артеріальної гіпертензії, а саме комбінованих таблеток, на основі антигіпертензивних речовин різних фармакологічних груп є вельми актуальним для вітчизняної фармацевтичної науки і практики. За результатами попередніх досліджень було розроблено склад і технологію комбінованих антигіпертензивних таблеток «Амлопамід». У якості діючих речовин таблетки мають амлодипіну безилат, лізиноприлу дигідрат та індапамід. У складі допоміжних речовин – мікрокристалічна целюлоза, лактози моногідрат, крохмаль картопляний і кальцію стеарат. Мета роботи – вивчення фізико-хімічних, фармако-технологічних показників та мікробіологічної чистоти комбінованих таблеток в процесі зберігання. Для визначення стабільності таблеток «Амлопамід», отриманих методом прямого пресування, були використані сучасні методи досліджень у відповідності до вимог ДФ України. Для встановлення терміну придатності таблеток та вивчення стабільності серії препарату були закладені на зберігання в різних упаковках при температурі (20±5)ºС. Контроль стабільності препарату проводили за наступними характеристиками: зовнішній вигляд, середня маса таблеток, час розпадання, розчинення, однорідність дозованих одиниць, ідентифікація та кількісний вміст діючих речовин, мікробіологічна чистота. Результати досліджень показали, що в процесі зберігання протягом 27 місяців при температурі (20±5)ºС зразки комбінованих таблеток «Амлопамід» в усіх використаних упаковках за всіма показниками відповідають вимогам нормативної документації та ДФУ. Встановлено термін придатності таблеток – 2 роки при зберіганні при кімнатній температурі.

ИЗУЧЕНИЕ СТАБИЛЬНОСТИ КОМБИНИРОВАННЫХ АНТИГИПЕРТЕНЗИВНЫХ ТАБЛЕТОК В ПРОЦЕССЕ ХРАНЕНИЯ

О.П.Стрилец

Ключевые слова: *комбинированные таблетки; амлодипин; индапамид; лизиноприл; срок хранения; стабильность*

Создание лекарственных средств для лечения артериальной гипертензии, а именно комбинированных таблеток, на основе антигипертензивных веществ разных фармакологических групп является очень актуальным для отечественной фармацевтической науки и практики. За результатами предыдущих исследований были разработаны состав и технология комбинированных антигипертензивных таблеток «Амлопамид». В качестве действующих веществ таблетки содержат амлодипина безилат, лизиноприла дигидрат и индапамид. В составе вспомогательных веществ – микрокристаллическая целлюлоза, лактозы моногидрат, крахмал картофельный и кальция стеарат. Цель работы – изучение физико-химических, фармако-технологических показателей и микробиологической чистоты комбинированных

таблеток в процессе хранения. Для определения стабильности таблеток «Амлопамид», полученных методом прямого прессования, были использованы современные методы исследований в соответствии с требованиями ГФ Украины. Для установления срока хранения таблеток и изучения стабильности серии препарата были заложены на хранение в разных упаковках при температуре (20±5)°С. Контроль стабильности препарата проводили по следующим характеристикам: внешний вид, средняя масса таблеток, время распадения, растворения, однородность дозированных единиц, идентификация и количественное содержание действующих веществ, микробиологическая чистота. Результаты исследований показали, что в процессе хранения на протяжении 27 месяцев при температуре (20±5)°С образцы комбинированных таблеток «Амлопамид» во всех используемых упаковках по всем показателям соответствуют требованиям нормативной документации и ГФУ. Установлен срок хранения таблеток – 2 года при хранении при комнатной температуре.

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

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THE STUDY OF THE CALENDULA FLOWERS EXTRACTION PROCESS

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Key words: tincture; Calendula officinalis; technology; pressure enhanced solvent extraction

This article presents the results of the study of the process of extraction of biologically active substances from the flowers of Calendula officinalis using pressure enhanced solvent extraction. The Timatic Micro laboratory extractor with the volume of 0.5 l designed for laboratory use when working with small amounts of the solvent and the product was used. The principle of its operation is based on a double action of pressure – low pressure and percolation of the plant raw material. It is possible to use various types of solvents (alcohols, water, glycerol, oils). The effect of the raw material particle size, the time of extraction, the number of cycles on quality parameters of the product has been studied. As a result of our studies, it has been found that the completeness of the BAS extraction is greatly influenced by the number of working cycles, as well as the time of the extraction process. The time of compression/decompression of the system was affected to a lesser extent. Thus, the optimal parameters of the calendula flowers extraction process are: extractant – 70% ethanol, degree of the plant raw material fineness – 3-5 mm, the ratio of the raw material to the extractant – 1:10, the extraction time – 5 hours, and the number of cycles – 75.

Calendula tincture is a well known drug that is in constant demand from buyers due to low prices and a wide range of the pharmacological action. Pot marigold possesses the anti-inflammatory, antibacterial, wound healing, spasmolytic, hypotensive and sedative properties, increases the metabolic function of the liver. A wide therapeutic activity of calendula is explained by the presence of a considerable number of carotenoids, flavonoids, salicylic acid, vitamins and other compounds [8-11].

At chemical and pharmaceutical enterprises Calendula tincture is obtained by simple maceration or percolation with 70° ethanol [1, 3].

These methods of tincture production do not meet modern requirements since the time required to obtain the finished product is long enough, and the plant raw material is exhausted only by 70% [2, 3, 5, 7].

Pressure enhanced solvent extraction takes the conventional solvent based extraction technology to a new level. This extraction technology effectively combines the use of solvents and elevated pressures to provide superior yields of high quality extracts from naturally occurring materials [4].

Extraction is carried out by the combined effect of compression/decompression and forced percolation of the solvent under pressure through the raw material layer. The extraction cycle alternates between dynamic phases obtained via a programmed pressure and static phases for transfer of the desired extract into the solvent [4, 6, 12].

Experimental Part

Calendula flowers (*Calendula officinalis L*) were selected as the study subjects [2, 10].

The process of extraction of calendula flowers by pressurized solvent extraction (PSE) was investigated, and the technology for obtaining of calendula tincture using a 0.5 l Timatic Micro extractor was developed.

The experiments on studying the effects of the particles size, the extraction time and the number of the working cycles on the yield of extractive substances were conducted.

To study the effect of the extraction time on the yield of extractives 50±0.5 g of the powdered calendula flowers was loaded in the extractor, filled with 70° ethyl alcohol (the ratio of the raw material to the extractant was 1:10), left for 1 hour for swelling and then extracted for 60, 120, 180, 240, 300, 360, 420 min. The parameters of the extraction process are shown in Tab. 1.

While studying the effect of the number of working cycles on the yield of extractives the following parameters of extraction given in Tab. 2 were used.

After extraction the resulting extract was drained and clarified for 48 hours in the refrigerator at 8-10°C. Then the extract was filtered, and a dry residue was obtained.

The samples obtained were tested according to the requirements specified in analytical normative documents (AND) [2, 10].

Results and Discussion

The completeness and speed of extraction of the active ingredients from the plant raw material depend on technological properties of the material, difference in concentrations, the time of extraction, the nature of the extractant and other factors, which should be considered in the extraction process.

The particle size affects the extraction process because it determines the phase interface between the material and the extractant. The effect of the degree of the raw material fineness on extraction of Calendula flowers was studied (Fig.).

The results showed that the most complete yield of extractive substances from the raw material was for par-

Table 1

The parameters of the extraction process

Equipment	Timatic Micro, 0.5 l; Filter bag, 100 mcm
The set parameters	Compression – 8 min, decompression – 8 min
	Temperature – 20°C, pressure – 5 bar
Time of extraction	1-6 hours
Calendula flowers	Humidity – 8±0.3%, particle size – 3-5 mm, 50±0.5 g each batch

Table 2

The parameters of the extraction process

No. of the experiment	The set parameters
1	Compression – 8 min, decompression – 5 min
2	Compression – 5 min, decompression – 5 min
3	Compression – 5 min, decompression – 2 min
4	Compression – 2 min, decompression – 2 min
	Temperature – 20±1°C, pressure – 5 bar
Time of extraction	4-6 hours
Calendula flowers	Humidity – 8±0.3%, particle size – 3-5 mm, 50±0.5 g each batch

ticles with the size of 1 mm (28.15±0.38%). However, excessive grinding leads to contamination by ballast substances and causes difficulties in the product purification. The raw material with the particle size of 3 to 5 mm has the value of this index with the difference in bias between 27.11±0.47% and 26.08±0.42%, respectively. Based on the results of the research we believe that to use the plant raw material with the particle size of 3-5 mm is the most optimal.

For our further studies a small Timatic extractor from Tecnolab (Perugia, Italy) was used. For industrial extractions the firm manufactures a larger Timatic extractor (200 l). The advantages of these extractors are their relatively low cost and further maintenance support by the manufacturing company.

Timatic is a solid-liquid extractor used for industrial production of herbal extracts. According to the manufacturer, percolation is achieved by alternating the dynamic phase, during which a pre-set pressure is generated, followed by the static phase, so that the solvent penetrates into the plant cells and releases again. The pressure phase prevents the formation of channels, as well as partial over-saturation of the solvent. Another advantage of this equipment is that it can be used for many different liquid solvents. It is semi-automatic, easy to handle, with a well designed display, with an automatic warning system and an automatic cleaning programme.

The effect of the extraction time on the yield of extractives. Selection of the optimum extraction time is required to obtain economically profitable tinctures on an industrial scale. The experimental results of studying the extraction time impact on the yield of extractives are shown in Tab. 3.

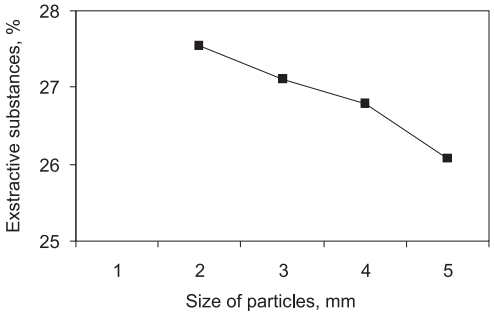

Fig. The yield of extractives of Calendula flowers depending on the size of particles.

Table 3

The effect of the extraction time on the yield of extractives

Time of extraction	Number of working cycles	Dry residue, %
60	4	0.94±0.03
120	8	1.02 ±0.04
180	11	1.13±0.05
240	15	1.25±0.04
300	19	1.31±0.05
360	22	1.48±0.03
420	26	1.56±0.03

The extraction rate was lower than expected. According to the requirements of AND the dry residue content in the calendula tincture should be not less than 1.7%. In the extracts obtained this parameter was in the range of 0.94-1.56%, and it did not satisfy the requirements. Of the possible explanations there is a rather small number of compression-decompression working cycles. The next step of our research was to study the effect of compression-decompression cycles on the yield of a dry residue.

The effect of the number of working cycles on the yield of extractives. The process parameters, i.e. the compression time, the decompression time and the number of cycles were varied (Tab. 2). The experimental results are shown in Tab. 4.

It was noted that of all parameters the number of working cycles had the greatest impact on the extraction process, while the time of compression/decompression

Table 4

The effect of the number of working cycles on the yield of extractives

Working cycle/ Time of extraction, h	Number of working cycles / Dry residue, %		
	240	300	360
1	19/ 1.29±0.04	23/ 1.55±0.04	27/ 1.76±0.03
2	24/ 1.51±0.03	30/ 1.76 ±0.05	36/ 1.95±0.03
3	35/ 1.68±0.04	43/ 1.96±0.05	51/ 2.17±0.04
4	60/ 1.88±0.05	75/ 2.21±0.04	90/ 2.28±0.05

Table 5

The results of determination of the calendula tincture quality indicators

Parameter	Calendula tincture	Requirements of AND
Description	A yellowish-brown liquid	a yellowish-brown liquid
Dry residue,%	2.21±0.05	not less than 1.7 %
Alcohol content,%	69.00±1.84	the final alcohol concentration is not less than 69 per cent of that of the initial extraction solvent
Heavy metals	Corresponds	not more than 0.001%
Thin-layer chromatography	Corresponds	according to the SPhU
Methanol and 2-propanol	Corresponds	maximum 0.05 % v/v of methanol and maximum 0.05 % v/v of 2-propanol
Microbiological purity	Corresponds	not more than 1,000 bacteria and 100 fungi

had no significant effect. Thus, the optimal parameters of the calendula flowers extraction process have been suggested; they are: extractant – 70% ethanol, degree of the plant raw material fineness – 3-5 mm, the ratio of the raw material to the extractant – 1:10, the extraction time – 5 hours, and the number of cycles – 75.

Thus, with such parameters of the technological process the time extraction (compared to 48 hours in conventional percolation) significantly reduces, and the yield of extractives increases by 5%

The tincture obtained was tested for compliance with the requirements of AND: description, the content of alcohol (not less than 65%), heavy metals, dry residue (not less than 2.1%) and microbiological purity. The results are presented in Tab. 5.

According to the research results the indicators of quality of the product obtained meet the requirements of AND.

CONCLUSIONS

The process of extraction of biologically active substances from the flowers of *Calendula officinalis* using pressure enhanced solvent extraction has been studied. The effect of the raw material particle size, the time of extraction, the number of cycles on quality parameters of the product has been determined. Thus, the optimal parameters of the calendula flowers extraction process are: extractant – 70% ethanol, degree of the plant raw material fineness – 3-5 mm, the ratio of the raw material to the extractant – 1:10, the extraction time – 5 hours, and the number of cycles – 75.

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ДОСЛІДЖЕННЯ ПРОЦЕСУ ЕКСТРАКЦІЇ КВІТОК КАЛЕНДУЛИ ПІД ТИСКОМ

Ю.В.Юдина

Ключові слова: *Календула лікарська; настойка; технологія; екстрагування під тиском*

Наведені результати дослідження процесу вилучення БАР з квіток календули лікарської з використанням інтенсифікованого методу екстракції під тиском. При роботі використовували лабораторний екстрактор Timatic Micro об'ємом 0,5 л, призначений для лабораторного використання при роботі з невеликими кількостями розчинника і продукту. Принцип його дії ґрунтується на подвійному впливі тиску – зниженні тиску і перколяції рослинної сировини. Можливе використання різних типів розчинників (спиртів, води, гліцерину, олії). Було вивчено вплив розміру частинок сировини, часу екстракції, числа робочих циклів на показники якості отриманого продукту. В результаті досліджень було встановлено, що на повноту вилучення БАР більший вплив чинить кількість робочих циклів, а також час процесу екстракції. Час компресії / декомпресії системи мав незначний вплив. При цьому були встановлені оптимальні параметри ведення процесу екстракції квіток календули: екстрагент – 70% етанол, ступінь подрібнення рослинної сировини – 3-5 мм, співвідношення сировини до екстрагенту 1:10, час екстракції – 5 годин, кількість робочих циклів – 75.

ИЗУЧЕНИЕ ПРОЦЕССА ЭКСТРАКЦИИ ЦВЕТКОВ КАЛЕНДУЛЫ ПОД ДАВЛЕНИЕМ

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Ключевые слова: *Календула лекарственная; настойка; технология; экстракция под давлением*

Приведены результаты исследования процесса извлечения БАВ из цветков календулы лекарственной с использованием интенсифицированного метода экстракции под давлением. Был использован лабораторный экстрактор Timatic Micro объемом 0,5 л, предназначенный для лабораторного использования при работе с небольшими количествами растворителя и продукта. Принцип его действия основан на двойном воздействии давления – понижении давления и перколяции растительного сырья. Возможно использование различных типов растворителей (спиртов, воды, глицерина, масла). Было изучено влияние размера частиц сырья, времени экстракции, числа рабочих циклов на показатели качества полученного продукта. В результате исследований было установлено, что на полноту извлечения БАВ большее влияние оказывает количество рабочих циклов, а также время процесса экстракции. Время компрессии/декомпрессии системы оказывало незначительное влияние. При этом были установлены оптимальные параметры ведения процесса экстракции цветков календулы: экстрагент – 70% этанол, степень измельчения растительного сырья – 3-5 мм, соотношение сырья к экстрагенту 1:10, время экстракции – 5 часов, количество рабочих циклов – 75.

Recommended by Doctor of Pharmacy, professor L.S.Strelnikov

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THE METHOD FOR OBTAINING OF THE PROTECTIVE PERTUSSIS ANTIGEN BY LOW-FREQUENCY ULTRASOUND

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Key words: ultrasound; fraction; antigen; vaccine; immunogenicity; Bordetella pertussis

This article describes the physical technology for obtaining surface antigens with protective properties from Bordetella pertussis microbial cells in the absence of additional use of chemical and synthetic substances. The mechanical destruction of cell membranes of microorganisms was carried out under mild conditions using low power low-frequency ultrasound, it allowed not to damage the protective bioactive substances. Ultracentrifugation of microbial ultrasound desintegrates and the subsequent gel – chromatographic separation allowed to obtain a protective antigen with the molecular weight of ~ 8.1 kDa and significantly enhance its specific weight from (62.5±9.1%) to (86.2±4.6%) (P<0.05). The study of toxicity in the test of the mice weight changes showed the presence of pathogenicity factors in the entire antigenic complex and in the fractionated component with the weight of ~ 3.0 kDa. And the purified native antigen with the weight of ~ 8.1 kDa in the calculated dose (160 mg) did not contain toxic compounds. The antigen with the molecular weight of ~ 8.1 kDa showed no histamine-sensitizing and dermonecrotic properties. While studying immunogenicity of the fraction with the weight of ~ 8.1 kDa a strong direct correlation was found between increase of vaccination antigen doses and the corresponding increase of the immunity intensity of vaccinated animals. It indicates the specificity of the results obtained and the protective activity of this fraction. The percentage of survived mice vaccinated by the native antigen with the molecular weight of ~ 8.1 kDa 1.9 times exceeded the percentage of animals vaccinated by the standard industry sample of pertussis vaccine.

Nowadays commercial drugs of acellular pertussis vaccines manufactured by various countries (Japan, France, UK, etc.) are usually obtained using chemical methods that have significant drawbacks: difficult set-up because of the necessity to carry out multistage operations of isolation and purification of bacteria cell structures, and the chemical structure of the isolated antigens can change under the influence of reagents.

Physical methods of antigens obtaining are considered as possible alternative technologies. They are attractive, first of all, by the fact that their implementation is achieved under standard conditions and avoids the need to remove extracting agents from the isolated composite structures of pathogenic bacteria. This opens the prospect of obtaining permanent components with a high protective activity. Ultrasound disintegration plays the major role in the mechanical destruction of the microorganism cell walls for extracting protective bioactive substances; it also allows to break microbial cells while keeping their intercellular content [5].

The aim is to create the technology for obtaining surface antigens with protective properties from pertussis pathogen microbial cells by ultrasound in the absence of additional use of chemical and synthetic substances.

Materials and Methods

Isolation of native pertussis antigens was carried out from the production strain of *Bordetella pertussis* No.267 provided by "Biolik" CJSC (now "Pharmstandard – Biolik" JSC) by the physical method. Destruction of microbial cells was performed by low-frequency ultrasound, followed by ultracentrifugation of desintegrates, filtration, concentration and fractionation of the antigenic complex by gel-chromatography (the equipment of the company LKB, Sweden).

The degree of disintegration of *B. perertussis* cells was controlled by the following parameters: decrease of the optical density of the microbial suspension was measured by Densi-La-Meter, and the total protein concentration was measured by Lowry (a set by "Simko LTD", Lviv) [6].

The biochemical analysis of the antigenic drugs obtained from pertussis pathogen microbial cells was carried out at the Department of Biochemistry of the Kharkiv National Medical University. In the filtrate the amount of total protein was determined by Lowry method. Then precipitation and hydrolysis of protein were performed. In the hydrolysate the content of carbohydrate compounds, lipids and nucleic acids was determined.

Toxicity of the antigen substances studied was evaluated according to the WHO recommendations and "Analytical documentation for the pertussis suspension" using the following tests:

1) the test of weight changes in mice with calculation of the absolute and relative increase of the body weight [1, 7-8];

2) the test on the presence of the dermonecrotic effect determined by intradermal introduction of the drugs studied to guinea pigs weighing 300 g and rabbits weighing 2 kg in the dose of 0.2 ml. The end result of

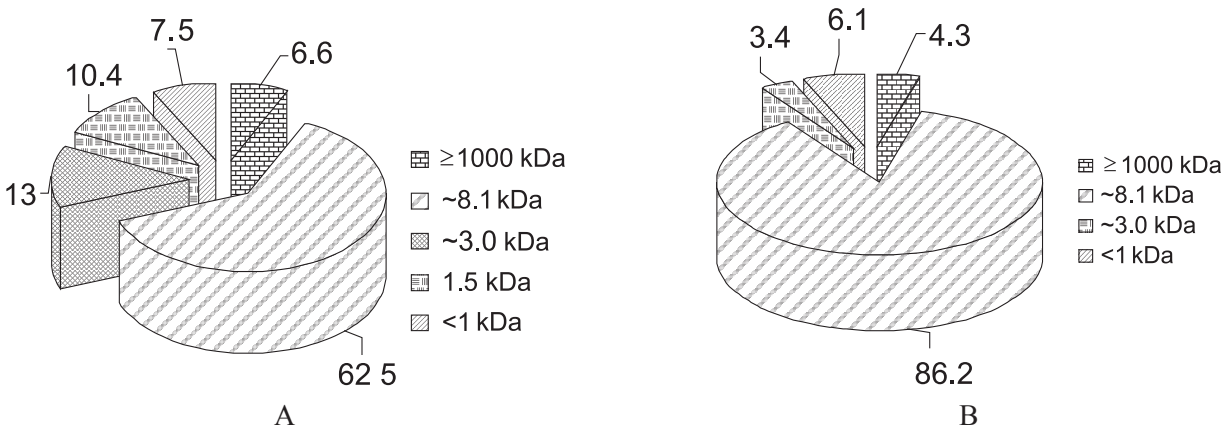

Fig. The specific weight of fractions with a different molecular weight by low-frequency ultrasound.
A – in the absence of additional ultracentrifugation of microbial disintegrates.
B – after ultracentrifugation of microbial disintegrates.

the experiment was assessed in 96 hours by the presence or absence of necrosis at the injection site [1];

3) the histamine-sensitizing test of purified antigens with calculation of HSD (the histamine-sensitizing dose) and HSD_{50} (the estimated dose sensitizing 50% of the animals) indices [1, 7-8].

Immunogenicity of the antigenic drugs under research was studied according to the WHO recommendations in intracerebral infection of animals with a virulent pertussis culture (strain No.18323) [7].

Experiments on animals were conducted in compliance with the requirements of the Directive of the European Parliament "On the protection of animals used for scientific purposes" (2010/63/EU), in accordance with the Declaration of Helsinki of the World Medical Association (WMA), recommendations of the code of ethics (1985) in the section "International Guiding Principles For Biomedical Research Involving Animals" and the Law of Ukraine "On protection of animals from cruel treatment" No.3447-IV (2006).

All experiments were repeated 3-4 times. Statistical processing of the experimental results was performed using the programme packages Excel 2003 and "Biostat-4". To characterize the reliability of the results obtained the parametric criteria were used with determination of the mean value (M) and its standard deviation (±m). To assess the reliability of differences between figures of the control and experimental groups the Student t-test correlation method was used [2-3].

Results and Discussion

The treatment of the *B.pertussis* microbial mass when using low-frequency ultrasound was 7 hours since exactly this exposure was considered to be enough to provide a significant result of disintegration of pertussis pathogen microbial cells: within the first 6 hours disintegration of the industrial strain did not occur, but longer treatment (8 hours) allowed to statistically unreliable increase the number of the isolated intracellular complexes.

Chromatographic fractionation of the disintegrated pertussis pathogen microbial mass allowed to isolate both high- and low-molecular complexes, among which antigens with the molecular weights of ~ 8.1 kDa dominated, and high-molecular antigens with the molecular weights over 1.000 kDa were present in small amount (Fig.).

Purification of the desintegrate by ultracentrifugation significantly affect the ratio of the isolated fractions. The fraction specific weight of ~ 8.1 kDa increased by 1.4 times (from 62.5±9.1 to 86.2±4.6, $P<0.05$), and the total percentage of fractions with the lower molecular weight reduced by 3.3 times, i.e. the ratio of fractions (≥1000 kDa, ~ 8.1 kDa, ≤3.0 kDa) before ultracentrifugation in relation to decrease of the molecular weight was 1:9.5:4.7, and after it – 1:20:2.2.

The chemical structure of the desintegrate, supernatant after centrifugation of the samples, as well as the corresponding samples of antigenic fractions with the molecular weights over 1000 kDa, ~ 8.1 kDa, ~ 3.0 kDa are represented as mixtures where proteins dominate; carbohydrates and lipids are found in much lower concentrations, and nucleic acids are present only in the hundredths parts of microgram. The antigenic fraction with the molecular weight of ~ 8.1 kDa contains 85% of protein, 7.9% of carbohydrates and 7.1% of lipids. Ultracentrifugation of the desintegrate affects their ratio in the faction. Thus, antigens with the molecular weight of ~ 8.1 kDa obtained by ultracentrifugation contained 1.3 times less carbohydrates and 7.5 times less nucleic acids. When comparing indicators of separate purified components some differences were found: in the fraction with the weight of ~ 8.1 kDa there were more protein structures (1.8 times), as well as lipids and carbohydrates (1.6-1.8 times) than in a high-molecular antigen with the weight of more than 1000 kDa.

When studying reactogenicity of the entire unfractionated antigen complex in the test of weight changes in mice with calculation of absolute and relative increase in the body mass there was the death of mice within 3 days of observation. It can indicate the presence of pathogenicity factors in this complex drug. After vaccination of laboratory animals with antigenic fractions with the molecular weight of ~ 8.1 kDa in the calculated dose (160 mcg) the increase in mice weight was (66.6±4.2) % of the weight increase of animals in the control group, i.e. within the requirements of nor-

mative documents for vaccine drugs [1]. The study of toxicity of the compound with the molecular mass of ~ 3.0 kDa showed that this fraction significantly inhibited the animal mass growth. It is assumed that during fractionation of the entire antigenic complex its toxic compounds remain in the low-molecular fractions (~ 3.0 kDa).

When studying the dermonecrotic activity of the fraction with the molecular weight of ~ 8.1 kDa, in the dose being 1.7 times higher than the calculated one the necrotic skin injuries of animals were not observed. It indicates the absence of the dermonecrotic factor in the given drug.

According to the WHO recommendations the histamine-sensitizing test reflects reactogenicity of acellular vaccines most accurately [7-8]. To compare the results the corpuscular pertussis vaccine (produced by "Biolik" CJSC) was used. As a control ISS-5 (the industry standard sample: pertussis vaccine calibrated by L.A.Tarasevich`s DISC according to the international standard) and saline solution (placebo) were used. The histamine-sensitizing of antigens with the molecular weight of ~ 8.1 kDa in the vaccinated dose containing 160 mcg of protein showed no sensitizing effect in mice: the average HSD index values were 16.7 times less 0.5 (control value of HSD index corresponding to the low-toxic level). These fractions showed a significantly lower histamine-sensitizing activity compared to other antigens and a corpuscular pertussis vaccine.

The methodical peculiarity of the study of immunogenic properties of antigenic fractions is introduction of the virulent culture of *Bordetella pertussis* No.18323 in the infected dose of 476.2 LD_{50} and increase of the number of mice in each experimental group from 16 to 20 according to the recommendations [4]. As a control ISSS-42-28-89-01 P (the industry standard sample) was used.

According to the results of studying immunogenicity of the fraction with the molecular weight of ~ 8.1 kDa it has been found that increase of the antigen dose leads to increase of the immunity intensity of the vaccinated animals. Thus, mice vaccination with the specified fraction in amounts of 25 mcg/dose, 18 mcg/dose and 12 mcg/dose causes the protective effect in (71.7±5.8)%, (26.7±5.7)% and (18.3±5.0)% cases, respectively (ρ – the rank correlation coefficient – 1). A strong direct correlation indicates the specificity of the results obtained and the protective activity of the faction specified. The percentage of survived mice vaccinated by the native antigen with the molecular weight of ~ 8.1 kDa 1.9 times exceeded the percentage of animals vaccinated by the standard industry sample of pertussis vaccine.

CONCLUSIONS

1. Disintegration of *B.pertussis* microbial cells when using low power low-frequency ultrasound has allowed identifying native chemically unmodified biologically-active substances. By chromatographic separation of the disintegrate the protective antigen with the molecular weight of ~ 8.1 kDa has been obtained in a dominant amount of (62.5±9.1%), and an additional ultracentrifugation has increased its specific weight by 1.4 times (86.2±4.6%), $P<0.05$.

2. The purified fraction with the weight of ~ 8.1 kDa has no toxic, dermonecrotic and histamine-sensitizing properties.

3. The antigenic component with the molecular weight of ~ 8.1 kDa in the dose of 25.0 mcg provides the survival of mice after their infection with virulent culture of *Bordetella pertussis* No.18323 in (71.7±5.8) % cases. It proves its protective properties.

4. Application of physical factors without chemicals while isolating surface antigens of *B. pertussis* microbial cells can serve as a benchmark in developing technologies for obtaining native prototypes of candidate-vaccines, which are not modified by extractants.

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СПОСІБ ОТРИМАННЯ НАТИВНОГО ПРОТЕКТИВНОГО КАШЛЮКОВОГО АНТИГЕНУ ЗА ДОПОМОГОЮ НИЗЬКОЧАСТОТНОГО УЛЬТРАЗВУКОВОГО ЧИННИКА

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Ключові слова: *ультразвук; фракція; антиген; вакцина; імуногенність; Bordetella pertussis*

Описана фізична технологія одержання поверхневих антигенів з протективними властивостями з мікробних клітин збудника кашлюка при відсутності додаткового застосування хімічних і синтетичних речовин. Механічне руйнування клітинних оболонок мікроорганізму здійснювалось в щадному режимі за допомогою низькочастотного ультразвуку малої потужності, що дозволило не пошкодити протективні біологічно активні речовини. Ультрацентрифугування мікробних ультразвукових дезінтеграторів та наступне гель – хроматографічне розділення дозволили отримати протективний антиген з молекулярною масою ~ 8,1 кДа та достовірно збільшити його питому вагу з (62,5±9,1%) до (86,2±4,6%) (P<0,05). Вивчення токсичності в тесті зміни маси мишей показало наявність факторів патогенності в цілому антигенному комплексі та у фракціонованому компоненті з масою ~ 3,0 кДа, а очищений нативний антиген з масою ~ 8,1 кДа в розрахованій дозі (160 мкг) не містив токсичних сполук. Антигенний компонент з молекулярною масою ~ 8,1 кДа не проявляв гістамін-сенсибілізуючих та дермонекротичних властивостей. При вивченні імуногенності фракції з масою ~ 8,1 кДа встановлено сильний прямий кореляційний зв'язок між збільшенням щеплювальної дози антигену та відповідним збільшенням напруженості імунітету у вакцинованих тварин, що вказує на специфічність одержаних результатів та протективну активність зазначеної фракції. Відсоток мишей, що вижили, яких вакцинували нативним антигеном з молекулярною масою ~ 8,1 кДа перебільшив відсоток тварин, щеплених галузевим стандартним зразком кашлюкової вакцини, в 1,9 рази.

СПОСОБ ПОЛУЧЕНИЯ НАТИВНОГО ПРОТЕКТИВНОГО КОКЛЮШНОГО АНТИГЕНА С ПОМОЩЬЮ НИЗКОЧАСТОТНОГО УЛЬТРАЗВУКОВОГО ФАКТОРА

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Ключевые слова: *ультразвук; фракция; антиген; вакцина; иммуногенность; Bordetella pertussis*

Описана физическая технология получения поверхностных антигенов с протективными свойствами из микробных клеток возбудителя коклюша при отсутствии дополнительного применения химических и синтетических веществ. Механическое разрушение клеточных оболочек микроорганизма осуществлялось в щадящем режиме с помощью низкочастотного ультразвука малой мощности, что позволило не повредить протективные биологически активные вещества. Ультрацентрифугирование микробных ультразвуковых дезинтеграторов и последующее гель-хроматографическое разделение позволило получить протективный антиген с молекулярной массой ~ 8,1 кДа и достоверно увеличить его удельный вес с (62,5±9,1%) до (86,2±4,6%) (P<0,05). Изучение токсичности в тесте изменения массы мышей показало наличие факторов патогенности в целом антигенном комплексе и во фракционированном компоненте с массой ~ 3,0 кДа, а очищенный нативный антиген с массой ~ 8,1 кДа в расчетной дозе (160 мкг) не содержал токсических соединений. Антиген с молекулярной массой ~ 8,1 кДа не проявлял гистамин-сенсибилизирующих и дермонекротических свойств. При изучении иммуногенности фракции с массой ~ 8,1 кДа установлена сильная прямая корреляционная связь между увеличением прививочной дозы антигена и соответствующим увеличением напряженности иммунитета у вакцинированных животных, что указывает на специфичность полученных результатов и протективную активность указанной фракции. Процент выживших мышей, которых вакцинировали нативным антигеном с молекулярной массой ~ 8,1 кДа превысил процент животных, привитых отраслевым стандартным образцом коклюшной вакцины, в 1,9 раза.

Recommended by Doctor of Pharmacy, professor O.I.Tikhonov

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DETERMINATION OF CRITICAL PARAMETERS OF PRODUCTION TECHNOLOGY FOR LESFAL

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Key words: liposomes; technology; critical parameters of production

Currently some success has been achieved when using liposomes in development of new drugs with various actions. Scientists have demonstrated the use of phospholipids as the raw material for production of liposomes. Supercritical fluids are used for producing multilayer, large and small monolayer liposomes. The size and behaviour of liposomes are determined primarily by the presence of a closed membrane wall. As a result, liposomes remain undamaged under various unfavourable conditions, and their membrane is capable to self-regenerate after its structural damages. A positive factor is that flexibility and fluidity of the bilayer give liposomes a high ductility. An important task in development of the liposomal drug technology is to determine the critical parameters that affect obtaining of stable, well characterized liposomal dispersions in large quantities. Critical parameters of the production technology of Lesfal liposomal drug for injection have been determined. It has been found that the most critical step is to obtain a lipid film. The main factors affecting formation of impurities in the process of producing liposomes of Lesfal drug for injection are time; temperature and the vacuum level. The critical parameters for these factors such as time (45 min), temperature (43°C), the vacuum level (not less than 14 gPa) have been determined. They are the optimal values for producing a lipid film for Lesfal liposomes.

Currently some success has been achieved when using liposomes in development of new drugs with various actions. Scientists have demonstrated the use of phospholipids as the raw material for production of liposomes [5, 13].

As a rule, in the technology of liposomal products a liposome membrane is formed from the same phospholipids found in the composition of biological membranes, i.e. phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine. It allows to achieve complete biocompatibility of liposomes [3, 12].

Various methods are used for liposome production. For example, liposomes can be produced by ultrasound, dehydration / rehydration, etc. According to the method of dehydration/rehydration, a lipid is dissolved in an organic solvent (chloroform, dichloromethane, methanol, or ethanol), then the solution is evaporated, for instance, in a rotary evaporator. Water and buffer solution are added to the lipid film formed on the wall of the evaporating flask resulting in multilayer liposomes. Monolayer liposomes are produced by subsequent ultrasonic treatment of the lipid suspension [6, 9, 11].

Lately, the technology of supercritical fluids has been applied for liposome production. These methods allow producing multilayer, large and small monolayer liposomes. The size and behaviour of liposomes are determined primarily by the presence of a closed membrane wall. Despite the molecular thickness (approximately 4 nm), the lipid bilayer is distinguished by the remarkable mechanical strength and flexibility. As a result, liposomes remain undamaged under various unfavourable conditions, and their membrane is capable to self-regenerate after its structural damages. A positive factor is that flexibility and fluidity of the bilayer make liposomes highly plastic [7, 8].

Taking the above into account an important objective for development of the technology of liposomal drugs is to determine the critical parameters affecting production of stable, well-characterized liposomal dispersions in large quantities [10]. Sterile medicines require particularly serious attitude to ensure quality.

Critical parameters for the technology of a drug for injection using essential phospholipids, which may influence on the strength of the lipid film, and, as a result, on reproducibility from batch to batch, functional characteristics and quality of the product, are:

- the mixing time;
- the rate of the mixer when Lipoid EPCS 100 and excipients are dissolved;
- the pH of solutions, the quantitative content of phosphatidylcholine, lysophosphatidylcholine and benzyl alcohol;
- the time and storage temperature of the drug solution before bottling;
- temperature at the stages of technological process;
- formulation under the nitrogen atmosphere;
- sterility of the primary package;
- aseptic filling of the drug.

The most critical stage is production of a lipid film [2].

The aim of the work is to determine critical parameters of the production technology of Lesfal liposomal drug for injection.

Materials and Methods

Ethanol determination was performed by gas chromatography (SPhU, 2.2.28) [4] on an Aglient 7890N gas

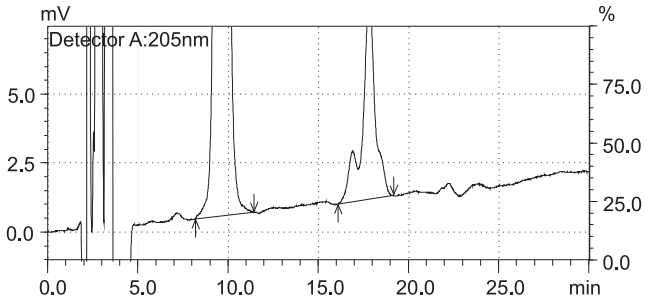

Fig. Integration of the peaks of phosphatidylcholine.

chromatograph with a flame-ionisation detector under the following conditions:

- a HP-INNOWax fused silica capillary column with the size of 30 m × 0.25 mm covered with a layer of polyethylene glycol with the layer thickness of 0.25 μm;
- the column temperature: 100°C;
- the injection temperature: 230°C;
- the detector temperature: 250°C;
- the split ratio: 1:25;
- the carrier gas: helium for chromatography *R*;
- the flow rate: 0.7 mL/min.

The content of ethanol in 1 mL of the drug (X_2), mg, was calculated using the equation:

$$X_2 = \frac{S_1 \cdot m_0 \cdot 1 \cdot 10 \cdot P}{S_0 \cdot 100 \cdot 1 \cdot 10 \cdot 100} = \frac{S_1 \cdot m_0 \cdot P}{S_0 \cdot 10000}, \qquad (1)$$

where: S_0 – is the average area of the peak of ethanol in the chromatogram obtained with *Reference solution* (b); S_1 – is the average area of the peak of ethanol in the chromatogram obtained with *Test solution*; m_0 – is the weighed amount of ethanol used for preparation of *Reference solution* (b), mg; P – is the content of the major substance in ethanol used for preparation of *Reference solution* (b), %.

Determination of phosphatidylcholine and lysophosphatidylcholine was performed by liquid chromatography (SPhU, 2.2.29) [4] on an Aglient 1200 liquid chromatograph with an ultraviolet detector under the following conditions:

- the steel column with the size of 250×4.6 mm packed with Kromasil Si sorbent with the particle size of 5 μm, or equivalent meeting the requirements of the chromatographic system suitability;
- the mobile phase: isopropanol–*n*-hexane–water *R* (67:16.5:16) ultrasonically degassed;
- the flow rate: 1.0 mL/min;
- detection: at 205 nm;
- the column temperature: 40°C;
- the volume of injection: 10 μL for determination of phosphatidylcholine; 50 μL for determination of lysophosphatidylcholine.

Before the analysis the chromatographic column was equilibrated with the mobile phase in the volume of not less than 300 mL.

Reference solutions (a) and (b) were chromatographed.

The retention time of the peak of phosphatidylcholine is about 10 min; the retention time of the peak of lysophosphatidylcholine is about 18 min. The peaks are integrated as shown in Fig. below.

The chromatographic system is considered to be suitable if:

- the number of theoretical plates calculated by the peak of phosphatidylcholine is not less than 1000;
- the relative standard deviation is not more than 2.0% calculated for 5 areas of the peak of phosphatidylcholine in the chromatogram obtained with *Reference solution* (a) and the peak of lysophosphatidylcholine in the chromatogram obtained with *Reference solution* (b).

The content of lysophosphatidylcholine in 1 mL of the drug (X_4), mg, was calculated using equation 2:

$$X_4 = \frac{S_1 \cdot m_0 \cdot 50 \cdot P \cdot \rho}{S_0 \cdot 100 \cdot m_1 \cdot 100} = \frac{S_1 \cdot m_0 \cdot P \cdot \rho}{S_0 \cdot m_1 \cdot 200}, \qquad (2)$$

where: S_0 – is the average area of the peak of lysophosphatidylcholine in the chromatogram obtained with *Reference solution* (b); S_1 – is the average area of the peak of lysophosphatidylcholine in the chromatogram obtained with *Test solution*; m_0 – is the weighed amount of lysophosphatidylcholine used for preparation of *Reference solution* (b), mg; m_1 – is the weighed amount of the drug used for preparation of *Test solution*, g; P – is the content of the major substance in lysophosphatidylcholine used for preparation of *Reference solution* (b), %; ρ – is the density of the drug.

The content of lysophosphatidylcholine per 1 mL of the drug should be:

- not more than 4 mg at the time of release;
- not more than 6.62 mg on the expiration date.

Evaporation of the alcoholic solution of Lipoid EPCS 100 was performed on a BUCHI rotary evaporator R-215 using the nitrogen blanketing. This rotary evaporator comprises the following major components: a vacuum controller, a flask rotation speed controller and a vapour temperature sensor, a water-bath, a vacuum pump. Temperature and the vacuum level are automatically maintained in the equipment.

Results and Discussion

During the experiment when evaporating the alcoholic solution of Lipoid EPCS 100 the following parameters were changed: 1) the time of evaporation at constant temperature and the vacuum level, 2) the temperature at vacuum of 20 gPa and the time of 45 min, 3) the vacuum level at constant temperature and time. The results of the study are presented in Tab. 1, 2, 3.

As shown by the data from Table 1, with the time of evaporation from 35 to 45 min the content of lysophosphatidylcholine impurity is within the allowed limit range, but the content of ethanol does not comply with the specification. The data from Tab. 2 indicate that the results are best approximated to the optimal ones at the temperature of 43°C and the time of 45 min, however, the content of lysophosphatidylcholine is at the upper limit, and the content of ethanol is too high. The required content of impurities in the drug is achieved at the given values of time, temperature, and at the vacuum level of 12 and 14 gPa (Tab. 3).

Table 1

Determination of the optimal time for production of a lipid film

Experiment No.	Temperature, °C	Vacuum, gPa	Time, min	Content of impurities (not more than 4.0 mg/mL)	Content of ethanol (not more than 5.02 mg/mL)
1	40	20	35	0.8	12.6
2	40	20	40	0.9	8.5
3	40	20	45	3.6	7.7
4	40	20	50	4.7	7.2
5	40	20	60	6.4	6.3

Table 2

Determination of the optimal temperature for production of a lipid film

Experiment No.	Temperature, °C	Vacuum, gPa	Time, min	Content of impurities (not more than 4.0 mg/mL)	Content of ethanol (not more than 5.02 mg/mL)
1	40	20	45	3.6	7.7
2	41	20	45	4.0	6.7
3	43	20	45	4.0	6.5
4	45	20	45	5.8	2.0
5	50	20	45	6.5	1.1

Table 3

Determination of the optimal vacuum for production of a lipid film

Experiment No.	Temperature, °C	Vacuum, gPa	Time, min	Content of impurities (not more than 4.0 mg/mL)	Content of ethanol (not more than 5.02 mg/mL)
1	43	20	45	4.0	6.5
2	43	18	45	4.2	5.8
3	43	16	45	4.3	5.0
4	43	14	45	3.3	4.2
5	43	12	45	3.3	3.0

Table 4

The optimal parameters for production of a lipid film

Temperature, °C	Vacuum, gPa, Minimum	Time, min	Content of impurities (not more than 4.0 mg/mL)	Content of ethanol (not more than 5.02 mg/mL)
43	14	45	3.3	4.2

The optimal parameters of production of a lipid film are given in Tab. 4.

Taking into account the physical and chemical properties of Lipoid EPCS the terminal sterilization of the drug is impossible. Sterilization was performed by filtration through a 0.1 μm filter, which is acceptable according to the requirements of the SPhU, article *Methods of Preparation of Sterile Products*. The drug was aseptically filled in the volume of 5.0 mL into dark-glass ampoules previously blown with sterile nitrogen [1].

The material of ampoules (glass of hydrolytic class 1) was selected according to the requirements of the SPhU, article 3.2.1 *Glass Containers for Pharmaceutical Use*.

The preliminary stability study of the drug has confirmed suitability of the primary package.

CONCLUSIONS

1. The major factors that affect formation of impurities when producing liposomes of Lesfal drug for injection are time, temperature and the vacuum level.

2. The critical parameters for these factors such as time (45 min), temperature (43°C), the vacuum level (not less than 14 gPa) have been determined. They are the optimal values for producing a lipid film for Lesfal liposomes.

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ВИЗНАЧЕННЯ КРИТИЧНИХ ПАРАМЕТРІВ ТЕХНОЛОГІЇ ОТРИМАННЯ ПРЕПАРАТУ «ЛЕСФАЛЬ»

Г.І.Борщевський

Ключові слова: *ліпосоми; технологія; критичні параметри виробництва*

У теперішній час досягнуті певні успіхи при використанні ліпосом у створенні нових лікарських препаратів різної спрямованості дії. Вченими доведено застосування фосфоліпідів в якості сировини для приготування ліпосом. Найбільш часто для отримання ліпосом використовують технологію надкритичних розчинів. За її допомогою можна отримати багатошарові, великі і дрібні одношарові ліпосоми. Розміри ліпосом і їх поведінка визначаються, насамперед, наявністю у них замкнутої мембранної оболонки. Внаслідок цього ліпосоми зберігають цілісність при різних несприятливих умовах, а їх мембрана має здатність до самовідновлення при структурних пошкодженнях. Позитивним фактором є те, що гнучкість бішару і його плинність надають ліпосомам високої пластичності. Важливим завданням при розробці технології ліпосомальних препаратів є визначення критичних параметрів, які впливають на отримання стабільних, добре характеризованих ліпосомальних дисперсій у великих кількостях. Визначені критичні параметри технології отримання ін'єкційного ліпосомального препарату «Лесфаль». Встановлено, що найбільш критичною є стадія отримання ліпідної плівки. Основними факторами, що впливають на утворення домішок у процесі отримання ліпосом ін'єкційного препарату «Лесфаль», є час; температура і рівень вакууму. Визначені критичні параметри даних факторів: час (45 хв), температура (43°С), величина вакууму (не менше 14 гПа), які є оптимальними для процесу отримання ліпідної плівки ліпосом препарату «Лесфаль».

ОПРЕДЕЛЕНИЕ КРИТИЧЕСКИХ ПАРАМЕТРОВ ТЕХНОЛОГИИ ПОЛУЧЕНИЯ ПРЕПАРАТА «ЛЕСФАЛЬ»

Г.И.Борщевский

Ключевые слова: *липосомы; технология; критические параметры производства*

В настоящее время достигнуты определенные успехи при использовании липосом в создании новых лекарственных препаратов различной направленности действия. Ученые доказали применение фосфолипидов в качестве сырья для приготовления липосом. Наиболее часто для получения липосом используют технологию сверхкритических растворов. С её помощью можно получить многослойные, крупные и мелкие однослойные липосомы. Размеры липосом и их поведение определяются, прежде всего, наличием у них замкнутой мембранной оболочки. В результате этого липосомы сохраняют целостность при различных неблагоприятных условиях, а их мембрана обладает способностью к самовосстановлению при структурных повреждениях. Положительным фактором является то, что гибкость бислоя и его текучесть придают липосомам высокую пластичность. Важной задачей при разработке технологии липосомальных препаратов является определение критических параметров, влияющих на получение стабильных, хорошо характеризуемых липосомальных дисперсий в больших количествах. Определены критические параметры технологии получения инъекционного липосомального препарата «Лесфаль». Установлено, что наиболее критичной является стадия получения липидной пленки. Основными факторами, влияющими на образование примесей в процессе получения липосом инъекционного препарата «Лесфаль», являются время; температура и уровень вакуума. Определены критические параметры данных факторов: время (45 мин), температура (43°С), уровень вакуума (не менее 14 гПа), которые являются оптимальными для процесса получения липидной пленки липосом препарата «Лесфаль».

UDC 615.07:615.2:543.544

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

QUALIFICATION OF TLC-EQUIPMENT USED IN ANALYSIS OF THE COMBINED HERBAL MEDICINES

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Key words: quality control system; analysis; TLC-equipment; Klimased

On the example of Klimased drug with the purpose of choosing the analytical equipment for quality control of combined drugs of the plant origin the study on major functional and performance characteristics (design qualification) of the equipment used according to pharmacopoeial requirements(SPhU and USP) and the target analytical tasks has been conducted. It allows to select equipment for an enterprise. A comparative analysis of functional and technical specifications of the devices to perform analysis of the combined drugs from the medicinal plant raw material by the method of thin-layer chromatography has been conducted. It has been experimentally proven that the configuration of the chromatographic chamber does not affect the result of the analysis, chambers manufactured by Sorbfil are more affordable by economic indicators. When analysing the medicinal plant raw material and drugs from it the TLC and HPTLC plates on all substrates with an UV indicator can be used, it is advisable to make the final choice by economic components or their availability. Characteristics of the devices for applying solutions of chromatograms have revealed that when choosing microsyringes the best indicator is its volume. When comparing devices for detecting or quenching fluorescence manufactured by Sorbfil (Russia) and Spectroline (USA) it has been proven that each of the devices studied can be used to identify chromatographic zones. The manufacturer Spectroline has the quality certificate ISO 9001:2008 and is more affordable, therefore, it is superior to irradiator UVS-254/365 manufactured by Sorbfil. Among the devices for spraying chromatograms the sprayers of GGB (USA) have a comfortable design and a suitable volume, but by cost they are much more expensive than sprayers of Sorbfil (Russia). It is advisable to make the final choice based on the economic component.

A key element in organization of production and quality management is the quality control system, and one of its main objectives is to obtain reliable results during the analysis. Such results are only possible when using laboratory equipment with the required technical characteristics and correct functioning.

Equipment qualification is a necessary preliminary step for validation/verification of analytical methods [1, 4, 8].

The aim of our paper was to qualify the laboratory equipment used in the analysis of a multicomponent herbal drug Klimased by thin-layer chromatography (TLC).

Materials and Methods

When evaluating the quality of Klimased drug according to its Drug Master File (DMF) such physical and physicochemical methods of analysis as TLC, HPLC, GC, etc., were used.

On the example of Klimased drug with the purpose of choosing the analytical equipment for its quality control the study of major functional and performance characteristics (design qualification) of the equipment used according to pharmacopoeial requirements (SPhU and USP) and the target analytical tasks were conducted. It allows to select equipment for an enterprise [2, 3, 6, 7, 9].

In the process of our study the propositions of manufacturers that supply analytical equipment used to identify the active substances of the drug under research (TLC) to the pharmaceutical market of Ukraine were analyzed.

Results and Discussion

The results of analysis of the requirements of the SPhU and USP to the equipment used in the TLC method in the qualitative analysis of Klimased are given in Tab. 1.

A comparative analysis of functional and technical specifications of the devices to perform analysis by the method of thin-layer chromatography is given in Tab. 2.

As Tab. 2 shows, a comparative analysis of functional characteristics of the chromatographic chambers of different manufacturers (Sorbfil, Russia and GGB, USA) has found that the size of the plate affects the choice of chambers for conducting the experiment. The Latch-LID Chromatotanks model (27×7×26 cm) manufactured by GGB (USA) is optimal when using the TLC plates with the size of 20×10 (for simultaneous analysis of many samples). It has been experimentally proven that the configuration of the chromatographic chamber does not affect the result of the analysis [5]. Chambers manufactured by Sorbfil are more affordable by economic indicators.

Comparative characteristics of analytical and high-performance plates for TLC of such manufacturers as Sorbfil (Russia), Macherey-Nagel (Germany), Merck (Germany) have found that as a sorbent all plates contain silica gel, which is selective in separation of compounds containing various functional groups of the medicinal plant raw material (the medicinal plant raw material and drugs with the medicinal plant raw material) [5, 6, 8]. The plates under research contain aluminium, polyethylene terephthalate, glass as a substrate, each of them has advantages (inert glass material, aluminium and polyethylene terephthalate are easy to use) [5, 6]. When ana-

Table 1

Qualification of equipment for TLC analysis of Klimased drug (requirements of the SPhU and USP)

Equipment	Requirements of the SPhU	Requirements of the USP
TLC or HPTLC plates	The stationary phase consisting of a suitable material applied in the form of a standardized thin layer and fixed on the base (plate) made of glass, metal or plastic	
	To use plates made in industrial conditions is permitted if they comply with the requirements of section 4.1.1. «Reagents», as well as "System suitability test" described in a separate article. It is recommended to determine convergence of R_f. values	To use plates made in industrial conditions is permitted
	The substrate is made of glass, metal or plastic, coated with a layer of silica gel with a suitable thickness and particle size for HPTLC plates – 2-10 μm and for common TLC plates – 5-40 μm. If necessary, the particle size is indicated after the name of the sorbent in the tests where it is used. The sorbent may contain an organic binding substance	The average particle size for HPTLC plates – 5 μm, for TLC plates 10-15 μm
Micropipettes, microsyringes, calibrated capillaries	Devices suitable for applying solutions	Manual, semi-automatic or automatic devices for applying samples. A template for drawing zones manually at specific intervals, measuring the distance and assisting in labelling can be additionally used
Chromatographic chamber	The container with a tight fitting lid and a flat bottom or a bottom with two troughs made of an inert transparent material corresponding to the size of the plates used	
Developing devices or reagents	Suitable devices used to transfer reagents on the plate by spraying, vapour treatment or immersion that provide, if necessary, heating to detect the compounds separated	
Devices for detecting or quenching fluorescence	Requirements are not given	The UV light source for examining in the short-wave (254 nm) and long-wave (365 nm) light
Documentation	For example, photographs or computer files can be used for documenting the chromatograms detected	A suitable device to register the chromatograms detected

Table 2

A comparative analysis of functional and technical specifications of the devices for TLC analysis and their cost

Equipment	Name	Description	Cost
1	2	3	4
Chromatographic chamber			
Sorbfil, Russia	Chromatographic chamber with a dividing ledge for plates of 10×10 cm. The size – (150×120×80 mm)	Chambers are made of chemically resistant glass. They have a dividing ledge at the bottom for fixing plates and saving the eluent. Chambers are supplied with a ground lid	2 000 RUB
	Chromatographic chamber for plates of 15×15 cm. The size – (150×120×80 mm)		2 850 RUB
	Chromatographic chamber for plates of 15×15 cm (glued with silicone). The size – (190×195×65 mm)		1 600 RUB
GGB, USA	Chromatographic chamber with a flat bottom (Latch-LID Chromatotanks). The size – (27×7×26 cm)	Heavy glass of the design is suitable for regular use for many years. The design is sustainable against accidental contact due to the weight (about 15 feet) and the flat bottom. A clear glass allows to inspect easily the contents of the chamber from all sides. A flat lid provides reliable fit, it is made of milk glass with edges beveled inside and outside avoiding all sharp edges. The unique design of self-locking has the corresponding latches on the lid and the chamber and allows the structure when closing the chamber with the lid be firmly in position. Metal components of the mechanism-latch lid are made of stainless steel	156.28 $

Table 2 continued

1	2	3	4
	Chromatographic chamber with a flat bottom, 10 cm Thinline (Latch-Lid Chromatotank unit/Tank – Lid, intended for plates of 10×10 cm or 10×5 cm. The size – (12×6.4×11.5)	This model can place up to two plates and is suitable for storing small amounts of test solvents (when selecting the optimal solvent in the process of studying the unknown substances). A smaller cubic size of the chamber makes it faster and more evenly saturate the atmosphere with solvents and use a smaller volume of the solvent. A clear glass allows to inspect easily the contents of the chamber from all sides. A flat lid provides reliable fit, it is made of milk glass with edges beveled inside and outside avoiding all sharp edges. The unique design of self-locking has the corresponding latches on the lid and the chamber and allows the structure when closing the chamber with the lid be firmly in position. Metal components of the mechanism-latch lid are made of stainless steel	93.89 $
TLC or HPTLC plates			
Sorbfil, Russia, Krasnodar	Sorbfil PTLC-P-A (10x10), 50 ps	Analytical, without an UV indicator. Particle size: 5-17 μm. The substrate material – PET film (polyethylene terephthalate)	1480 RUB
	Sorbfil PTLC-P-A (10x15), 50 ps		2200 RUB
	Sorbfil PTLC-P-A (10x20), 50 ps		3350 RUB
	Sorbfil PTLC-P-A-UV (10x10), 50 ps	Analytical, with an UV indicator (254 nm). Particle size: 5-17 μm. The substrate material – PET film	1680 RUB
	Sorbfil PTLC-P-A-UV (10x15), 50 ps		2 580 RUB
	Sorbfil PTLC-P-A-UV (10x20), 50 ps		3 800 RUB
	Sorbfil PTLC-P-V (10x10), 50 ps	High-performance, without an UV indicator. Particle size: 8-12 μm. The substrate material – PET film	2100 RUB
	Sorbfil PTLC-P-V (10x15), 50 ps		3 300 RUB
	Sorbfil PTLC-P-V-UV (10×10), 50 ps	High-performance, with an UV indicator. PTLC-P-V-UV. Particle size: 8-12 μm. The substrate material – PET film	2 300 RUB
	Sorbfil PTLC-P-V-UV (10x15), 50 ps		3 590 RUB
	Sorbfil PTLC-AF-A (10×10), 50 ps	Analytical, without an UV indicator. Particle size: 5-17 μm. The substrate material – aluminium foil	1480 RUB
	Sorbfil PTLC-AF-A (10×15), 50 ps		2200 RUB
	Sorbfil PTLC-AF-A (10×20), 50 ps		3 350 RUB
	Sorbfil PTLC-AF-A-UV (10×10), 50 ps	Analytical, with an UV indicator (254 nm). Particle size: 5-17 μm. The substrate material – aluminium foil	1680 RUB
	Sorbfil PTLC-AF-A-UV (10×15), 50 ps		2 580 RUB
	Sorbfil, PTLC-AF-A-UV (10×20), 50 ps		3 800 RUB
	Sorbfil, PTLC-AF-V (10x10), 50 ps	High-performance, without an UV indicator. Particle size: 8-12 μm. The substrate material – aluminium foil	2100 RUB
	Sorbfil, PTLC-AF-V (10x15), 50 ps		3 300 RUB
	Sorbfil, PTLC-AF-V-UV (10×10), 50 ps	High-performance, with an UV indicator. Particle size: 8-12 μm. The substrate material – aluminium foil	2 300 RUB
	Sorbfil, PTLC-AF-V-UV (10×15), 50 ps		3 590 RUB
Macherey-Nagel, Germany	DC-Fertigfolien ALUGRAM Sil G/UV$_{254}$ (20×20) , 25 ps	Analytical, with an UV indicator (254 nm). Particle size: 5-17 μm. The substrate material – aluminium foil	1300 UAH
Merck, Germany	Silica gel 60 F$_{254}$ (10×10), 25 ps	Analytical, with an UV indicator (254 nm). Particle size: 10-12 μm. The substrate material – glass	1868.0 UAH
	Silica gel 60 F$_{254}$ (10×20), 50 ps	Analytical, with an UV indicator (254 nm). Particle size: 10-12 μm. The substrate material – glass	2347.0 UAH
	Silica gel 60 F$_{254}$ (20×10), 50 ps	High-performance, with an UV indicator (254 nm). Particle size: 5-6 μm. The substrate material – glass	4585 UAH

Table 2 continued

1	2	3	4
Device for applying			
Sorbfil, Russia	M-1, volume – 1 µl, without guiding (1 ps)	Calibration microsyringes are designed for metered applying standard solutions and dosed analytical samples on the plates. The needle has a brushed straight cut	2 750 RUB
	M-1H, volume – 1 µl, with guiding (1 ps)		3 570 RUB
	M-10, volume – 10 µl, without guiding (2 ps)		3 740 RUB
	M-10H, volume – 10 µl with guiding (2 ps)		5 170 RUB
	M-50, volume – 50 µl, without guiding (1 ps)		4 000 RUB
Hamilton, Switzerland	705N, volume – 50 µl	A microsyringe with a fixed needle, caliber 22s (22s/51/2)	574.50 UAH
	701N, volume – 10 µl,	A microsyringe with a fixed needle, caliber 26s (26s/51/2)	4 000 RUB
	702N, volume – 25 µl,	A microsyringe with a fixed needle, caliber 22s (22s/51/2)	470.50 UAH
Devices for detecting or quenching fluorescence			
Spectroline, USA	CM-10, a cabinet with 4W combined lamp (365 nm with 300µW/cm^2, 254 nm with 310 µW/cm^2). The size: 22.9×30.5×16.5 cm. The weight: 3.2 kg	The multifunctional cabinet that is specifically designed for use with Spectroline UV lamps. It is made of aluminium coated with polyurethane lacquer. A protective coating against UV-radiation is built in the vinyl window for looking at chromatograms. The vinyl window increases contrast between fluorescence area and the background, reducing eye fatigue. The cabinet can be ordered separately or with 4-/ 6-cotton lamp, that absorbs in the short-wave and long-wave regions	450 $
	CM-10 a cabinet with 6W combined lamp (365 nm with 350µW/cm^2, 254 nm with 390 µW/cm^2). The size: 22.9×30.5×16.5 cm. The weight: 3.4 kg		510 $
Sorbfil, Russia	Irradiator UVL -254/365 The supply voltage: 220 V Power consumption, VA: not more than 30 The size: 32×20×27 cm. The weight: 5 kg	The emission source: Luminescent ultraviolet lamp KLC 9/UV 365 nm – 1 ps Mercury germicidal lamp DKB 9 254 nm –1 ps The size of TLC plates is not more than – 15×15 cm	13023.00 UAH / 38800 RUB
Developing devices			
Sorbfil, Russia	Sprayer with a bulb, Sorbfil. The sizes: height – 170 mm, diameter of the sprayer – 26-30 mm.	A sprayer is intended for applying a developing reagent on chromatographic plates. A glass atomizer of the sprayer contains system and container for solution in the same body and placed on a PVC bulb	280.80 UAH / 1200 RUB
GGB, USA	Sprayer unit, volume – 10 ml	A screw design prevents «reverse spraying»; made of borosilicate glass. Changeable sprayer. The opportunity of choosing a sprayer of the appropriate volume. The device operates at low air pressure	117.75 $
	Sprayer unit, volume – 50 ml		117.75 $
	Sprayer unit, volume –125 ml		117.75 $

lysing the medicinal plant raw material both analytical (TLC) and high-performance (HPTLC) plates can be used, they differ mainly in the size of the particles. During routine analysis the use of TLC plates with the particle size of 5-17 µm is enough. The presence of an UV indicator is important when choosing plates since in the pharmaceutical analysis of various classes of biologically active substances (BAS) of the medicinal plant raw material detection of many compounds occurs in UV light. Therefore, when analysing the medicinal plant raw material and drugs from it the TLC and HPTLC plates on all substrates with a UV indicator can be used. It is advisable to make the final choice based on the economic component or availability of plates.

Characteristics of the devices for applying solutions of chromatograms have revealed that when choosing microsyringes the best indicator is its volume. The volume of 10-50 µl is optimal for the analysis of the medicinal plant raw material.

When comparing devices manufactured by Sorbfil (Russia) and Spectroline (USA) for detecting or quenching fluorescence it has been shown that each of the devices studied can be used to identify chromatographic zones. The manufacturer Spectroline has the quality certificate

ISO 9001:2008 and is more affordable, therefore, it is superior to an UVS-254/365 irradiator manufactured by Sorbfil.

Comparative characteristics of the devices for spraying chromatograms (Tab. 2) have found that sprayers of GGB (USA) have a comfortable design and a suitable volume, but by cost they are much more expensive than sprayers of Sorbfil (Russia). It is advisable to make the final choice based on the economic component or availability of devices.

CONCLUSIONS

On the example of Klimased drug with the purpose of choosing the analytical equipment for quality control of combined drugs of the plant origin the major functional and performance characteristics (design qualification) of the equipment used according to pharmacopoeial requirements (SPhU and USP) and the target analytical tasks has been studied. It allows to select equipment for an enterprise.

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КВАЛІФІКАЦІЯ ТШХ-ОБЛАДНАННЯ, ЩО ЗАСТОСОВУЄТЬСЯ В АНАЛІЗІ КОМБІНОВАНИХ РОСЛИННИХ ПРЕПАРАТІВ

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Ключові слова: *система контролю якості; фармакопейний аналіз; ТШХ-обладнання; Клімасед*

На прикладі препарату Клімасед з метою вибору аналітичного обладнання для проведення контролю якості комбінованих лікарських засобів рослинного походження досліджені головні функціональні і експлуатаційні характеристики (кваліфікація дизайну) обладнання, яке використовується відповідно до фармакопейних вимог (ДФУ і USP) і поставлених аналітичних завдань, що дозволяє провести вибір обладнання для підприємства. Проведено порівняльний аналіз функціональних і технічних характеристик приладів для виконання аналізу комбінованих препаратів з лікарської рослинної сировини методом тонкошарової хроматографії. Експериментально доведено, що конфігурація хроматографічної камери не впливає на результат аналізу, за економічним показником більш доступними є камери виробництва Сорбфіл. При аналізі лікарської рослинної сировини (ЛРС) і препаратів з ЛРС можуть бути використані ТШХ- і ВЕТШХ-пластинки на всіх підложках з УФ-індикатором, остаточний вибір доцільно проводити за економічною складовою чи їх доступністю. Характеристика пристроїв для нанесення розчинів хроматограм виявила, що оптимальним показником при виборі мікрошприців є його об'єм. При порівнянні пристроїв для виявлення або гасіння флуоресценції виробництва Сорбфіл (Росія) і Spectroline (США) встановлено, що будь-який з досліджуваних приладів може бути використаний для виявлення хроматографічних зон. Виробник Spectroline має сертифікат якості ISO 9001:2008 і за ціною більш доступний, що є його перевагою над опромінювачем УФС-254/365 виробництва Сорбфіл. Серед пристроїв для обприскування хроматограм зручну конструкцію і потрібний об'єм мають пульверизатори GGB (США), але за вартістю вони є значно дорожчими за пульверизатор Сорбфіл (Росія). Остаточний вибір доцільно проводити за економічною складовою.

КВАЛИФИКАЦИЯ ТСХ-ОБОРУДОВАНИЯ, ПРИМЕНЯЕМОГО В АНАЛИЗЕ КОМБИНИРОВАННЫХ РАСТИТЕЛЬНЫХ ПРЕПАРАТОВ

В.К.Яковенко, Е.А.Хохлова

Ключевые слова: *система контроля качества; анализ; ТСХ-оборудование; Климасед*

На примере препарата Климасед с целью выбора аналитического оборудования для контроля качества комбинированных лекарственных препаратов растительного происхождения

проведены исследования по изучению основных функциональных и эксплуатационных характеристик (квалификация дизайна) оборудования, которое используется в соответствии с требованиями фармакопей (ДФУ и USP) и поставленным аналитическим заданиям, что позволяет провести выбор оборудования для предприятия. Проведен сравнительный анализ функциональных и технических характеристик приборов для выполнения анализа комбинированных препаратов из лекарственного растительного сырья методом тонкослойной хроматографии. Экспериментально доказано, что конфигурация хроматографической камеры не влияет на результат анализа, по экономическим показателям более доступными являются камеры производства Сорбфил. При анализе ЛРС и препаратов из ЛРС могут быть использованы ТСХ- и ВЭТСХ-пластинки на всех подложках с УФ-индикатором, окончательный выбор целесообразно проводить по экономической составляющей или их доступности. Характеристика приборов для нанесения растворов хроматограмм показала, что оптимальным показателем при выборе микрошприца является его объем. При сравнении приборов для обнаружения или гашения флуоресценции производства Сорбфил (Россия) и Spectroline (США) показано, что каждый из исследуемых приборов может быть использован для выявления хроматографических зон. Производитель Spectroline имеет сертификат качества ISO 9001:2008 и по цене более доступен, что является его преимуществом перед облучателем УФС-254/365 производства Сорбфил. Среди приборов для опрыскивания хроматограмм удобную конструкцию и подходящий объем имеют пульверизаторы GGB (США), но по стоимости они значительно дороже пульверизаторов Сорбфил (Россия). Окончательный выбор целесообразно проводить по экономической составляющей.

ОРГАНІЗАЦІЯ ТА ЕКОНОМІКА ФАРМАЦІЇ

Recommended by Doctor of Pharmacy, professor A.S.Nemchenko

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THE STUDY OF APPROACHES TO RISK MANAGEMENT IN PHARMACY

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Key words: risk-management; risk sources; objects of influence of risk; pharmaceutical organizations

The article is devoted to peculiarities of risk management in pharmacy. The sources and objects of the risk with their division into internal and external ones have been studied. For each of the risk factors those components of the organization being affected at first have been defined. Therefore, the internal factors include the level of management, the stage of the life cycle of the organization, its financial condition, production and innovation potential, the quality management system, etc. The external factors include the impact of public health policies, domestic and international law, the political and economic situation in the country, etc. The data of literature regarding methods of risk management has been summarized. The methods, which are proposed for use in risk management, have been determined. Thus, the most common version of the classification, which is the most appropriate for practice activity, includes evasion, dispersion, compensation and location. In our opinion, limitation as a way to manage certain forms of risk should be added.

Risk management is aimed at reducing the level of losses associated with economic risks. Methods and measures for prevention and management of unexpected situations are based on the results of planning and economic activities of the organization, determination and risk assessment, economic analysis of the potential, the internal and external environment of the enterprise, the current legislation. Therefore, risks are an integral part of the enterprise's strategy, and they deserve special attention for study [2-8].

In view of the aforesaid the aim of our study is the peculiarities of risk management in pharmacy.

Materials and Methods

The objects of the research are theoretical and methodological principles of risk management in the pharmaceutical branch. General scientific and special methods of the system analysis and the analytical method were used in our work.

Results and Discussion

All the factors determining the level of risk are usually divided into two groups – objective (external) and subjective (internal) ones [2, 9, 10]. Objective factors are not connected directly with a particular enterprise. Subjective factors directly characterize the company. These groups of factors are closely related and interact with one another. For risk assessment and decision-making it is necessary to have full information regarding the internal and external environment and risk carriers. According to the abovementioned the analysis, which allows obtaining information about the sources of risk, conducting their identification and classifying them by the degree of impact, is carried out. The sources of risks associated with the activity of pharmaceutical organizations are shown in Fig. 1.

Each of these factors has a specific influence on the organization, refers to different areas and has different frequency of manifestations, and most importantly – different degrees of impact. The following table discloses the relationship of risk sources and objects which are under their influence (Table).

In real business situations various ways of risk management affecting all directions of activity of the company can be used. Despite the large number of publications on this problem there is no unified approach to the classification of methods of risk management among experts [2, 3, 4, 6, 7, 11-17]. One of the variant that are used in business practice is division of risk management methods into four types: avoidance, minimization, diversification, and limitation. Another variant of the classification includes the following kinds: evasion, dissipation, compensation and localization. In our opinion, it is advisable to use the second variant, but also to include the method of limitation, which comprises setting the maximum volume of commercial transactions per a counterparty, the maximum size of stocks, the maximum time limit or loan amount provided to the counterparty, the maximum amount of the borrowed funds. This addition is because the aforementioned method is widely used by pharmaceutical companies as a crisis management measure. The most common methods used in pharmaceutical organizations are presented in Fig. 2.

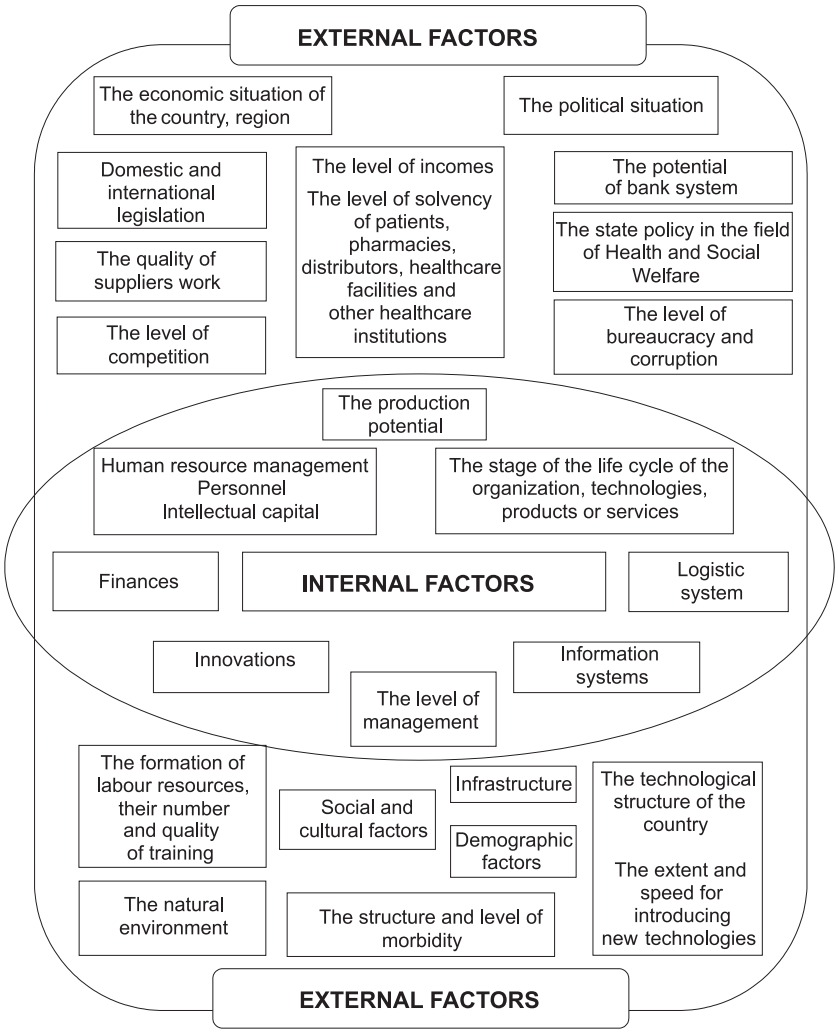

Fig. 1. The sources of key risks.

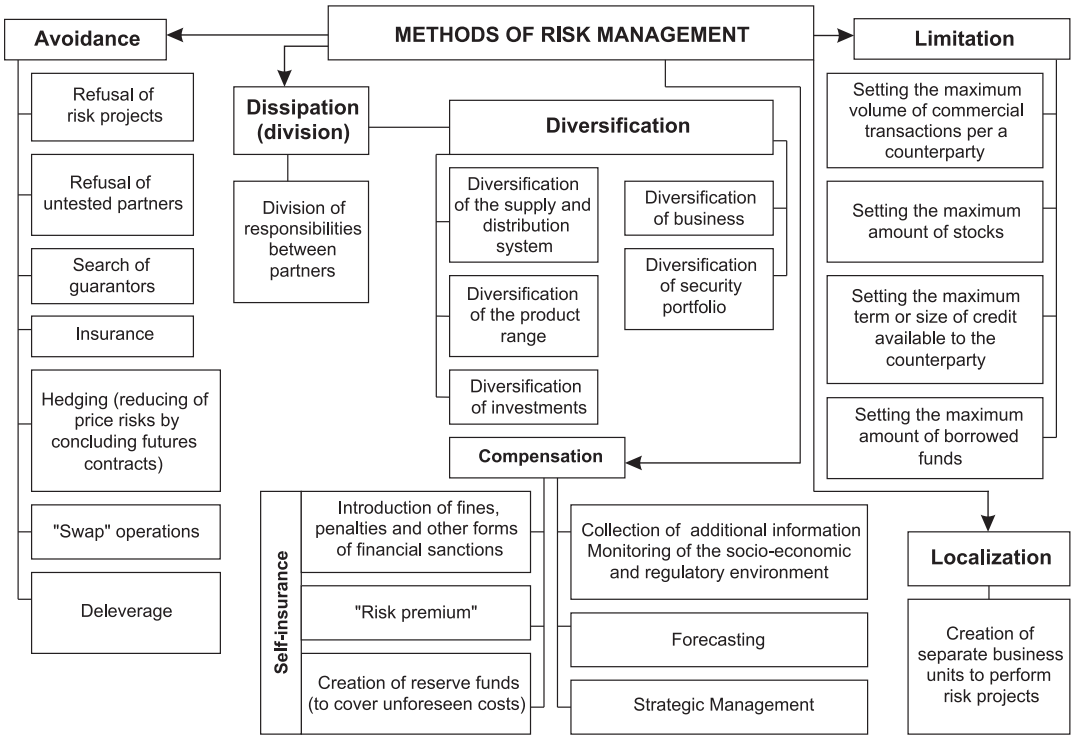

Fig. 2. Methods of risk management.

Table

Typical risk sources and objects for a pharmaceutical organization

Typical sources of risk	The object of the risk impact
Internal factors	
The production potential	Performance indicators; assets; resources; terms and schedule; ecology
Personnel	Organization resources; quality of products; standards of the customer service; terms and schedule; reputation
Intellectual capital	Income, performance; the level of introduction of new technologies; the product policy; reputation
Finances	Income, profit, the property status, costs, staff; terms and schedule, successful management performance (survival, effectiveness); solvency; resources
The stage of the life cycle of the organization, technologies, products or services	Financial and economic indicators, income; costs
Logistic system	Costs, the customer service standards, reputation, commercial secret
Information systems	The stability and efficiency of the subsystems of the organization; intangible assets (reputation)
The level of management	Quality of products and services; performance management; professional liability
Innovations	Profit, performance, intangible assets (reputation); ecology
External factors	
The level of incomes	Income; the assortment and product policy; the pricing policy
The level of solvency patients, pharmacies, distributors, healthcare facilities and other healthcare institutions	Income; the assortment and product policy; the pricing policy
The economic situation of the country, region	Gains; income; expenditure (for activities); staff; performance; solvency of the organization; resources
The level of bureaucracy and corruption	Gains; successful management indicators (existence and survival)
Legislation	Profit; costs; staff; resources; indicators of economic performance; successful management indicators (existence and survival)
International law	Gains; expenditure (for activities); staff; the product policy; the pricing policy; the manufacturing and innovative potential of the organization
The political situation	Gains; expenditure (for activities); performance management success (survival)
The quality of suppliers' work	The production subsystem of the organization; the logistic subsystem organization; intangible assets (reputation)
The potential of bank system	The innovation potential; solvency of the organization; costs; indicators of economic performance; successful performance management
The state policy in the field of Health and Social Welfare	Gains; successful performance management (effectiveness)
The level of competition	Gains; indicators of economic performance; successful performance management (effectiveness)
Social and cultural factors	Income; the product and assortment policy
The formation of labour resources, their number and quality of training	Gains; the innovation potential; the intellectual capital; indicators of economic performance; successful performance management (effectiveness); intangible assets (reputation)
The technological structure of the country	Innovation potential; indicators of economic performance
Infrastructure	Gains; staff; indicators of economic performance; successful performance management (effectiveness)
The structure and level of morbidity	Income; the product and assortment policy
Demographic factors	Income; the product and assortment policy
The natural environment	The assets; resources; performance; ecology

Risk management can be carried out on the basis of specially created programmes. After identifying negative trends and factors a complex of measures that are used for correction of the business unit activity should be developed. The above methods of risk management are the basis for formation of this complex. The final step is to compare the results of risk management with predicted indicators, i.e. controlling of risk management.

CONCLUSIONS

Summarizing the data the conclusion can be made that the system ensuring stability of the organization existence in difficult market conditions should have such component as risk management within its general structure.

The key conclusions:

1. The structure of sources of risk for a pharmaceutical organization with their division to internal and external ones has been created.

2. The relationship of risk sources and objects (components and indicators of the organization's activity) being under their influence has been shown.

Further refinement of the list of objects is the basis for development of risk management standards in the pharmaceutical business.

3. The literature data regarding the methods of risk management have been summarized. Methods of risk management for a pharmaceutical company have been determined.

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ВИВЧЕННЯ ПІДХОДІВ ДО УПРАВЛІННЯ РИЗИКОМ У ФАРМАЦІЇ

О.М.Євтушенко

Ключові слова: *ризик-менеджмент; джерела ризику; об'єкти впливу ризику; фармацевтичні організації*

Статтю присвячено особливостям ризик-менеджменту у фармації. Досліджені джерела та об'єкти впливу ризику з розподілом їх на внутрішні та зовнішні. Для кожного з ризикоутворювальних факторів відокремлені ті показники та складові організації, що підпадають під вплив у першу чергу. Так, до внутрішніх факторів відносять рівень менеджменту, етап життєвого циклу організації, її фінансовий стан, виробничий та інноваційний потенціал, систему управління якістю тощо. До зовнішніх факторів відносять вплив державної політики в галузі охорони здоров'я, вітчизняне та міжнародне законодавство, політичний та економічний стан країни тощо. Узагальнені дані літератури відносно методів управління ризиками. Визначені методи, що пропонуються для використання в системі управління ризиками фармацевтичної організації. Так, найчастіше зустрічається і є більш адекватним до практичної діяльності варіант класифікації, який включає: ухилення, дисипацію, компенсацію та локалізацію та до якого, на наш погляд, слід додати лімітування як інструмент, що дозволяє управляти певними формами ризику.

ИЗУЧЕНИЕ ПОДХОДОВ К УПРАВЛЕНИЮ РИСКОМ В ФАРМАЦИИ

Е.Н.Евтушенко

Ключевые слова: *риск-менеджмент; источники риска; объекты влияния риска; фармацевтические организации*

Статья посвящена особенностям риск-менеджмента в фармации. Исследованы источники и объекты воздействия риска с распределением их на внутренние и внешние. Для каждого из рискообразующих факторов выделены те составляющие организации, которые подвергаются влиянию в первую очередь. Так, к внутренним факторам относят уровень менеджмента, этап жизненного цикла организации, ее финансовое состояние, производственный и инновационный потенциал, систему управления качеством и др. К внешним факторам относят влияние государственной политики в области здравоохранения, отечественное и международное законодательство, политическое и экономическое положение страны и т. п. Обобщены данные литературы относительно методов управления рисками. Определены методы, предлагаемые для использования в системе управления рисками фармацевтической организации. Так, чаще всего встречается и является наиболее адекватным для практической деятельности такой вариант классификации, который включает в себя уклонение, рассеивание, компенсацию и локализацию, и к которому, на наш взгляд, следует добавить лимитирование как инструмент, позволяющий управлять определенными формами риска.

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ANALYSIS OF THE ASSORTMENT OF ANTIDIABETIC DRUGS AT THE PHARMACEUTICAL MARKET OF UKRAINE

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Key words: diabetes; drugs; assortment; marketing research; pharmaceutical market

The marketing research of antidiabetic drugs presented at the pharmaceutical market of Ukraine has been conducted. The product range of the group of antidiabetic drugs has been studied according to the ATC classification, manufacturing countries and dosage forms. According to the results of the analysis 49 drugs based on insulin and its analogues for injection, and 157 oral hypoglycemic drugs have been characterized. It is noted that insulins are unevenly divided by the duration of action. Most insulin drugs are presented by human insulin. Oral hypoglycemic drugs are characterized by a high saturation within subgroups. Drugs of aldose reductase inhibitors (Isodibut under the trade name Isodibut®) and other medicines, including 8 herbal medicines, are also used for treatment of diabetes. It has been found that 68% of antidiabetic drugs are imported from 21 countries of the Eurasian and South American continents. India occupies the leading positions. A wide range of antidiabetic drugs is presented by Germany, Poland, France, Denmark and Italy. The range of domestic drugs for treating diabetes is formed by 12 manufacturers. Domestic manufacturers offer only generic replacement of some active substances. At the pharmaceutical market of Ukraine antidiabetic drugs are available in the form of tablets, granules, powders, solutions and suspensions for injection. The research performed has shown that the market of antidiabetic drugs is characterized by heterogeneity of product groups, high concentration and monopolization of production, low competition and a small share of production of drugs attributable to domestic producers.

Diabetes is recognized as infectious epidemic of the XXI-th century, it takes the third place of the world's prevalence after cancer and cardiovascular diseases. The number and prevalence of people with diabetes is increasing rapidly. According to the International Diabetes Federation (IDF) in 2013 about 381.8 million people in the world had diabetes, and till 2035 this index will increase by 55% up to 591.9 million [6]. Recent data indicate that people in the countries with low and middle income represent the largest share of the epidemic (80%), and this disease affects much more people of the working age than previously thought. The largest number of people with diabetes is of the age from 40 to 59 years. According to the WHO data, two new cases of diabetes are diagnosed every six seconds, and one person dies because of its complications [8, 11]. In 2013, diabetes caused about 5.1 million of deaths [12]. According to the WHO in 2030 diabetes will be the seventh leading cause of death [9, 13].

According to the Ministry of Public Health of Ukraine in 2013 the number of patients with diabetes was over 1.3 million people, 212 134 of them require daily injections of insulin [2]. Increase in prevalence of diabetes in Ukraine reached 26% in the last 5 years [5]. A significant growth in the number of new registered cases of diabetes (primary disease) of the Ukrainian population is also observed: from 194.8 per 100 thousand of the population in 2005 to 249.8 in 2010, i.e. 23.7% within last 5 years. However, the number of patients is increasing mainly due to diabetes mellitus type 2 [4].

In connection with the abovementioned our country intensively searches not only more effective methods of diagnosis and treatment, but also more perfect organizational methods of treatment. It creates the opportunity to reduce the incidence of adverse long-term complications on the basis of improving detection of diabetic patients. Nowadays, one of these activities being of a special attention is the rational use of modern methods of treatment and extension of the range of antidiabetic drugs [15].

Materials and Methods

The aim of this study was to conduct the marketing analysis of the range of antidiabetic drugs presented at the pharmaceutical market of Ukraine. The object of the study was the information concerning the market structure of antidiabetic drugs registered in Ukraine. The graphical methods and analysis are used in the article.

To solve this goal the study of a conjuncture of domestic market of antidiabetic drugs was conducted. According to the results of the analysis the assortment of medicines of the Ukrainian pharmaceutical market was described.

Results and Discussion

According to the international ATC classification antidiabetic drugs are referred to group A "Medicines affecting the digestive system and metabolism" and constitute a subgroup A10 "Antidiabetic drugs" [3]. According to the State Register of Medicines of Ukraine [1] it has been found that as of 01.10.2014 antidiabetic drugs comprise 206 trade names (without regard to the amount in the pack) among 12 745 medicines registered in Ukraine. This group includes 49 drugs based

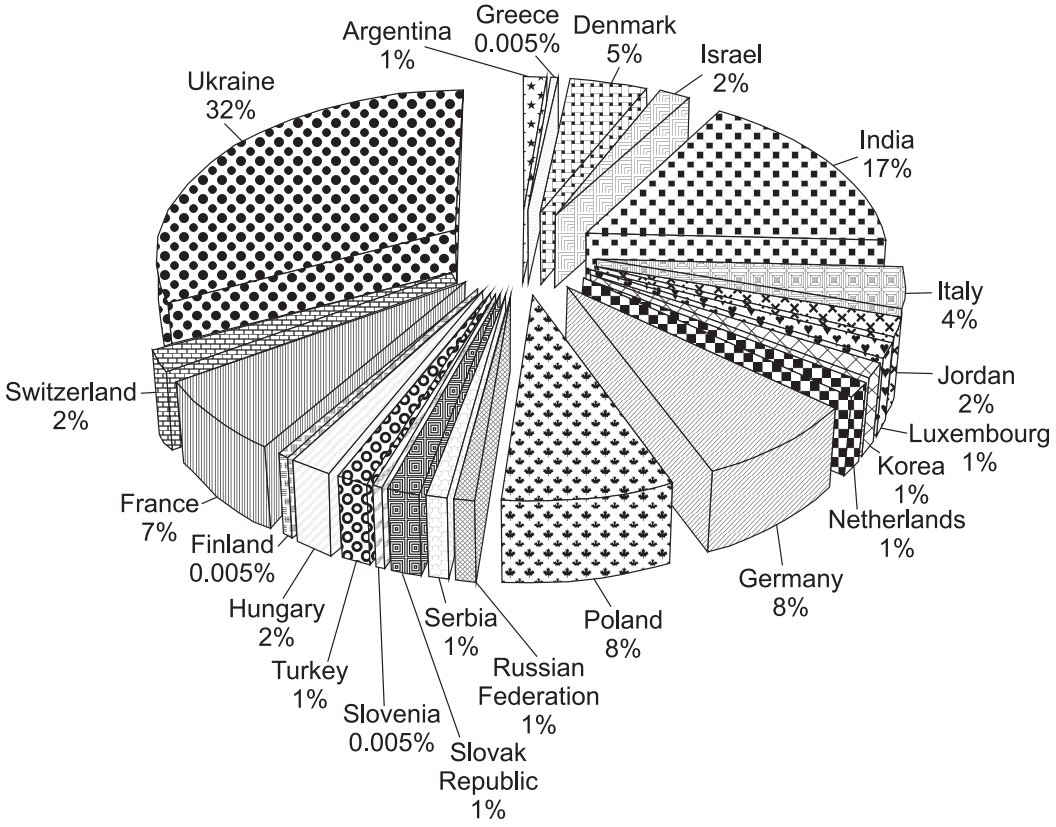

Fig. 1. The diagram of the ratio of antidiabetic drugs depending on the manufacturing country.

on insulin and its analogues for injection and 157 oral hypoglycemic drugs.

Only some representatives of each ATC-group for treating diabetes are registered at the pharmaceutical market of Ukraine. It should be noted that insulins are unevenly divided by the duration of action: 16 short-acting drugs, 14 drugs of the medium duration, and only 4 long-acting insulin medicines [14]. There are also 15 combinations of insulins with a short and medium duration of action for injection at the market. The overwhelming majority of all groups of insulins are presented by human insulin. Groups of insulin with a short and medium duration of action are characterized by individual representatives of porcine insulin (Monodar®), insulin lispro (Humalog®) and insulin asparagine (Novorapid® FlexPen®). Long-acting insulins and their analogues for injection are presented by 3 drugs based on insulin glargine and one on insulin detemir (Levemir® FlexPen®).

Oral hypoglycemic drugs are characterized by more saturation within subgroups. There are 38 drugs based on metformin at the Ukrainian pharmaceutical market of biguanides. The drugs of glimepiride (about 50 names) are the widest presented as derivatives of carbamide. There is a sufficiently wide assortment of drugs based on gliclazide (16 positions). Sulfonamides are presented by 5 drugs of glibenclamide and 1 of gliquidone (Glyurenorm®). There are often combinations of oral hypoglycemic drugs [7] such as metformin and sulfonamides (12 medicines), metformin with sitagliptin (Yanumet™) and metformin with vildagliptin (GalvusMet®). Thiazolidinediones are presented by 10 drugs on the basis of pioglitazone. Among the inhibitors of dipeptidyl peptidase-4 (dpp-4) [10] sitagliptin (Januvia™) appears three times, there is one vildagliptin (Galvus®), and saxagliptin (Ongliza) is presented twice. Other hypoglycemic drugs, excluding insulins, also include drugs of guar gum (Guarem), repaglinide (NovoNorm®) and liraglutide (Victoza®). Drugs of aldose reductase inhibitors (Isodibut under the trade name Isodibut®) and other medicines, including 8 herbal medicines (based on fruits of Saint-Mary-thistle, the valves of the bean fruit, blueberry shoots or the mixture of crushed medicinal plant raw material), are also used to treat diabetes.

The results of the study of the assortment structure of antidiabetic drugs indicate that 68% of drugs are imported from 21 countries. Geography of manufacturing countries is quite extensive and includes the countries of the Eurasian and South American continents (Fig. 1). India occupies the leading positions and supplies 35 drugs of the group studied to Ukraine. A wide assortment of antidiabetic drugs is presented by Germany (16 trade names), Poland (16 names), France (14), Denmark (10) and Italy (8).

Data of the analysis in Fig. 1 indicate that the share of the Ukrainian producers of antidiabetic drugs is 32%. However, most subgroups of antidiabetic drugs have no domestic analogues. The range of domestic drugs for treating diabetes is formed by 12 manufacturers.

It should be noted that in the list of drugs registered for treatment of diabetes distribution between manufacturing countries differs within the group. Thus, insulin and analogues for injection accounted 59.18% of the study group range and are characterized by a small

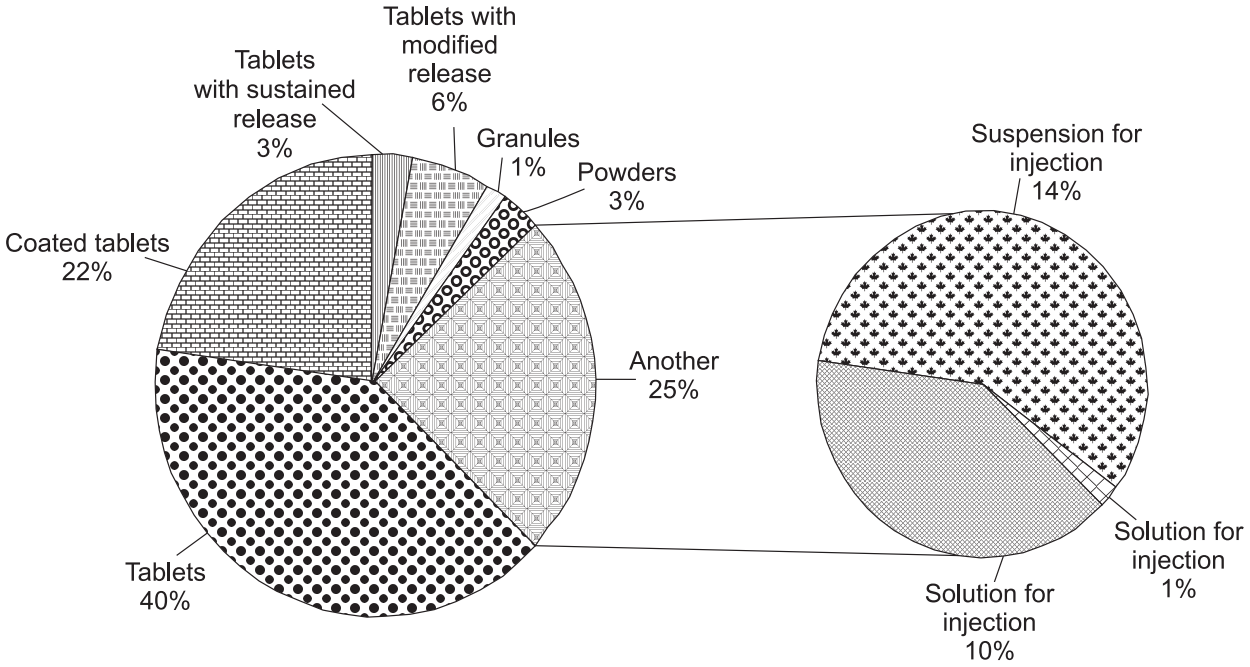

Fig. 2. Propositions of dosage forms of antidiabetic drugs at the pharmaceutical market of Ukraine.

number of countries. They are imported from of Denmark ("A/T Novo Nordisk" – 3), Poland ("Bioton S.A." – 3), Germany ("Sanofi-Aventis Deutschland GmbH" – 3). In addition, 3 finished medicinal products are manufactured by "Sanofi-Aventis Deutschland GmbH" (Germany), and "Pharma Life" Ltd. (Lviv, Ukraine), which is responsible for quality control and batch release, provides the secondary packaging. "Biocon Limited" (India) cooperates with "Darnitsa pharmaceutical company" JSC and supplies 3 drugs of this group at the Ukrainian market. The license holder of 2 insulin drugs manufactured by JSC "National biotechnology" (Russian Federation) is a domestic enterprise "UkrTerra LLC" (Kyiv, Ukraine). The Ukrainian insulin drugs and analogues for injection are manufactured by: "Indar" PJSC for production of insulin (7 drugs under such trade names as Humodar®, Monodar®), "Farmak" OJSC (Farmasulin® H – 6 drugs), "Darnitsa pharmaceutical company" JSC (Insugen-R (Regulyar) Insugen-N (NPH), Insugen-30/70 (Bifazik) – 4), Pharmaceutical company "Zdorovye" Ltd. (Gensulin R-Zdorovya, Gensulin H-Zdorovya and Gensulin M30-Zdorovya – 3). There are no domestic analogues in groups of drugs containing insulin lispro, insulin asparagine, insulin glargin, insulin detemir.

Oral hypoglycemic drugs are mainly supplied at the Ukrainian market from abroad. The largest part of imported drugs for treatment of type 2 diabetes is offered by India, these are 32 drugs. A large number of drugs are offered by several companies from around the world, mainly from the European region, for which a high level of development of the pharmaceutical industry is typical. Poland takes 8.28% of the market segment of oral hypoglycemic drugs, Germany – 6.37%, Italy and France – 5.10% each, Switzerland and Hungary – 2.55% each. The Jordanian firm "Al-Hikma Pharmaceuticals" PLC manufactures drugs based on glimepiride (Glianov®). Drugs of metformin hydrochloride are imported in Ukraine by the Israel manufacturer "TEVA Pharmaceutical Industries Ltd". Three trade names of drugs are imported from Argentina, Luxembourg, Korea, Netherland, Slovakia, Turkey. "Galenika a.d." supplies 2 drugs from Serbia. Oral hypoglycemic drugs are imported in Ukraine from Greece, Denmark, Slovenia, Finland.

Along with highly effective medications the patients are deprived of the opportunity to take other drugs since there is a monopoly of some imported drugs. Domestic manufacturers offer only generic replacement of some active substance. The Ukrainian enterprises produce only 46 drugs on the basis of metformin hydrochloride, glibenclamide, gliclazide, glimepiride, a combination of metformin hydrochloride and glibenclamide, pioglitazone, isodibut and the medicinal plant raw material. Groups of isodibut and other drugs are formed only by domestic producers. The domestic enterprises producing drugs of these groups are: "Farmak" OJSC, Kyiv; "Kusum Pharm" LLC, Sumy; "Technologist" PJSC, Uman, Cherkasy region; "Pharmex Group" LLC, Boryspil, Kyiv region; "Pharma Start" LLC, Kyiv; Pharmaceutical company "Zdorovye" Ltd., Kharkiv; "Indar" PJSC for production of insulin, Kyiv; "Luhansk Chemical and Pharmaceutical Plant" JSC, Luhansk; "Liktravy" PJSC, Zhitomir; Pharmaceutical factory "Viola" PJSC, Zaporizhzhya; "Lubnypharm" JSC, Lubny, Poltava region.

At the pharmaceutical market of Ukraine antidiabetic drugs are available in a variety of dosage forms such as tablets, granules, powders, solutions and suspensions for injection. A tablet form is presented by common tablets, coated tablets, tablets with sustained release and tablets with modified release. All insulins and analogues for injection are produced in the form of solutions or suspensions. The results of analysis of the drug assortment presented in Fig. 2 indicate the dis-

tribution of drugs by the dosage form and the route of administration taking into account the number of proposals for this form at the market.

Drugs of insulins and analogues for injection are available in two dosage forms. The suspension for injection is 59.18% of the assortment, 40.82% is in the form of the solution for injection. The suspension for injection is dispensed in bottles (17 positions), cartridges a carton box (20), cartridges inserted in multi-dosage disposable syringe pen (7). Among the solutions for injection 16 short-acting insulins and 4 long-acting drugs are proposed. Most of the drugs are produced simultaneously in several forms of packaging: in bottles – 9 trade names, in cartridges – 10, in cartridges built in sealed disposable syringe pens – 5 and in syringe pens – 3.

Among the oral hypoglycemic drugs registered the solid dosage forms quantitatively prevail: tablets constitute 93.63%, granules – 1.91%, powders – 3.82%, while the liquid dosage forms are only 0.64%. This is due to ease of use and accuracy of dosing of tablets. Currently, there is a clear tendency to increase the efficiency of pharmacotherapy in the treatment of type 2 diabetes. Offers of tablet hypoglycemic drugs are divided between tablets (83 drugs), coated tablets (46), tablets with sustained release (6) and tablets with modified release. Twelve names of tablets based on gliclazide are produced with modified release of the active substance.

In a powder form there are 6 drugs based on the medicinal plant raw material, including the valves of the bean fruit, blueberry shoots, and 4 herbal teas. Granules are presented by 3 trade names. Guarem is produced in 5 g granules in packs and 500 g containers with a measuring spoon. Granules from fruits of Saint-Mary- thistle are produced under the brand names of Silysem® and Gipoglisil®. They are dispensed in single-dose bags, single-dose coupled bags and in jars with a measuring spoon. At the same time, hypoglycemic drugs are produced in the form of a solution for injection. Thus, Victoza® is offered in cartridges inserted in pre-filled multi-dosage disposable syringe pen and in filled syringe pens in a carton box.

Thus, the studies conducted have shown that the market of antidiabetic drugs is characterized by heterogeneity of the product lines, high concentration and monopolization of manufacture, low competition and a small share of production of domestic drugs.

CONCLUSIONS

1. The marketing research of antidiabetic drugs presented at the pharmaceutical market of Ukraine has been conducted, and the structure of the product groups registered in Ukraine has been described.

2. The market of drugs for treating diabetes has been characterized according to the pharmacotherapeutic groups, manufacturing countries and dosage forms.

3. Quantitative and qualitative diversity of the current assortment of antidiabetic drugs presented by foreign companies and domestic manufacturers has been determined.

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АНАЛІЗ АСОРТИМЕНТУ АНТИДІАБЕТИЧНИХ ПРЕПАРАТІВ НА ФАРМАЦЕВТИЧНОМУ РИНКУ УКРАЇНИ

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Ключові слова: *діабет; лікарські засоби; асортимент; маркетингові дослідження; фармацевтичний ринок*

Проведені маркетингові дослідження антидіабетичних лікарських засобів, представлених на фармацевтичному ринку України. Вивчено товарний асортимент групи антидіабетичних засобів згідно з АТС-класифікацією, країнами виробниками та лікарськими формами. За результатами аналізу охарактеризовано 49 препаратів на основі інсулінів і аналогів для ін'єкцій та 157 пероральних гіпоглікемічних лікарських засобів. Відмічено, що за тривалістю дії інсуліни розподілені нерівномірно. Більшість препаратів інсуліну представлена інсуліном людським. Пероральні гіпоглікемічні лікарські засоби характеризуються більшим насиченням у межах підгруп. Для лікування цукрового діабету також використовують препарати інгібіторів альдозоредуктази (ізодибут під торговою назвою «Ізодибут®») та інші засоби, що включають 8 фітопрепаратів. Встановлено, що 68% препаратів антидіабетичних лікарських засобів імпортуються з 21 країни світу Євразійського та Південно-Американського континентів. Лідируючі позиції посідає Індія. Широкий асортимент антидіабетичних препаратів представлено з Німеччини, Польщі, Франції, Данії та Італії. Асортимент вітчизняних лікарських засобів для лікування цукрового діабету формують 12 фірм-виробників. Вітчизняні виробники пропонують лише генеричні заміни деяких діючих речовин. На фармацевтичному ринку України антидіабетичні препарати пропонуються у формі таблеток, гранул, порошків, розчинів та суспензій для ін'єкцій. Проведені дослідження показали, що ринок антидіабетичних лікарських засобів характеризується неоднорідністю асортиментних груп, високою концентрацією і монополізацією виробництва, низькою конкуренцією та незначною часткою випуску препаратів, яка припадає на вітчизняних виробників.

АНАЛИЗ АССОРТИМЕНТА ПРОТИВОДИАБЕТИЧЕСКИХ ПРЕПАРАТОВ НА ФАРМАЦЕВТИЧЕСКОМ РЫНКЕ УКРАИНЫ

О.В.Тригубчак

Ключевые слова: *диабет; лекарственные средства; ассортимент; маркетинговые исследования; фармацевтический рынок*

Проведены маркетинговые исследования противодиабетических лекарственных средств, представленных на фармацевтическом рынке Украины. Изучен товарный ассортимент группы противодиабетических средств согласно АТС-классификации, стран-производителей и лекарственных форм. По результатам анализа охарактеризовано 49 препаратов на основе инсулина и аналогов для инъекций и 157 пероральных противодиабетических лекарственных средств. Отмечено, что по продолжительности действия инсулины разделены неравномерно. Большинство препаратов инсулина представлены инсулином человеческим. Пероральные гипогликемические лекарственные средства характеризуются большим насыщением в пределах подгрупп. Для лечения сахарного диабета также используют препараты ингибиторов альдозоредуктазы (Изодибут под торговым названием «Изодибут®») и другие средства, включающие 8 фитопрепаратов. Установлено, что 68% препаратов противодиабетических лекарственных средств импортируются из 21 страны мира Евразийского и Юго-Американского континентов. Лидирующие позиции занимает Индия. Широкий ассортимент противодиабетических препаратов представлен из Германии, Польши, Франции, Дании и Италии. Ассортимент отечественных лекарственных средств для лечения сахарного диабета формируют 12 фирм-производителей. Отечественные производители предлагают только генерические замены некоторых действующих веществ. На фармацевтическом рынке Украины противодиабетические препараты предлагаются в форме таблеток, гранул, порошков, растворов и суспензий для инъекций. Проведенные исследования показали, что рынок противодиабетических лекарственных средств характеризуется неоднородностью ассортиментных групп, высокой концентрацией и монополизацией производства, низкой конкуренцией и незначительной долей выпуска препаратов, приходящейся на отечественных производителей.

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

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DEVELOPMENT OF STANDARD OPERATING PROCEDURES AND THEIR INTRODUCTION IN COMPOUNDING PHARMACIES

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Key words: standard operating procedure; compounding preparations; quality assurance of medicines

In accordance with the requirements of good manufacturing practices a proper keeping of documentation in compounding pharmacies licensed to prepare medicines should be an integral part of the quality assurance system and be a key element at all stages of preparation and quality control of compounding preparations. One of the important elements of proper documentation is the practice of using standard operating procedures (SOPs). The aim of this article is to describe the methodology of the SOP developing for licensed compounding pharmacies. Standard procedures should be developed according to the current regulatory framework and research. SOPs are developed by the staff involved in introduction of this procedure. SOPs should be reviewed by responsible persons and approved by the head. The following SOP structural elements have been identified: the title page (the name of institution, the name of the SOP, the classification number, the SOP version, the date on which the SOP enters into force, the signature of the responsible person); the goal; the scope of application; responsibility; stages of the procedure; revision (the term when the SOPs should be reviewed). The organizational structure of the quality assurance system is proposed for the SOP systematization: quality assurance, personnel, facilities and equipment, documentation, technology of preparation, quality control, carrying out works under the contract, complaints and recall, self-inspection. For each of the sections it has been proposed to develop SOPs focused on ensuring compliance with the procedure and to provide the quality aspect, which is the subject in this section.

According to the GPP requirements a good documentation in compounding pharmacies is an integral part of the quality assurance system and is a key element at all stages of preparation and quality control of compounding preparations (CP) [1, 2, 6, 13]. The purpose of existence and introduction of a good documentation must be determination, management, control and recording of all activities that may directly or indirectly affect all aspects of the CP quality [6]. The important element of a good documentation is the practice of using standard operating procedures (SOPs). The development of SOPs provides the requirements for preparation of the CP [1, 2], but it is not widely introduced into practice of compounding pharmacies of Ukraine.

According to the international experience in GPP introduction SOPs and working instructions should be developed for works and services carried out and available in compounding pharmacies, and which may affect the quality of public service and CP [7-10]. The special attention is paid to development of step-by-step procedures of compounding, quality control, labeling, storage of the CP, cleaning, disinfection, qualifications and operation with the equipment, etc. [10, 11]. The necessity of the SOP development and introduction in the compounding pharmacies of Ukraine has been confirmed by the results of the survey [5, 14].

The introduction of SOPs has a number of advantages. It helps to understand the process better, to optimize the working time and avoid additional unnecessary actions; to define duties of each employee and make sure that every employee knows them; to allow coordinating the implementation and sequence of the various stages of work, it provides proper execution of procedures at all stages of preparation, quality control, patient care, etc. SOPs can be also used as an educational tool for temporary workers, part-time workers, as the material for training of new employees, interns, and trainees. The SOP complex introduction should provide quality of the final product, confidence and coordination of operations. The SOP may contain additional information that is necessary for the self-inspection or audit. Standardization of the in-house quality control procedures requires the special attention [5, 14]. In the work [4] some aspects of the SOP development for retail pharmacies are given, approaches for development of the structure, the content and registration are discussed, as well as the list of typical SOPs is formed. For compounding pharmacies of Ukraine the question of the SOP development has been insufficiently studied; therefore, the additional studies are important.

The aim of this article is to describe the methodology of the SOP developing for licensed compounding pharmacies of Ukraine.

Materials and Methods

The study was performed using modern literature sources, the regulatory framework of Ukraine, data of pharmacopoeias and materials of own research. The methods of analysis, synthesis and data compilation were used in the work.

Results and Discussion

SOP development. While writing the SOP the author should use the current regulatory framework (Laws of

Table

The list of typical SOPs for compounding pharmacies

Name of the section	Examples and subjects of SOPs
Quality assurance	General procedures; the main stages of organizing the quality assurance system and good pharmaceutical practice, quality control; administrative responsibilities; writing the SOPs
Personnel	Staff recruitment; training; administrative responsibilities; the order to provide information to the public; consultations of physicians; CP dispensing; occupational hygiene
Facilities and equipment	The care procedure in terms of compounding, quality control, storage of CP; cleaning; registration of temperature and humidity in the premises; compliance with the sanitary and epidemiological requirements; purchase, maintenance, cleaning of the equipment; provision of analytical equipment operation
Documentation	The procedure for filling and storage of documentation
Technology of preparation	Preparation of active substances and excipients; compounding operations; obtaining of purified water; preparation of glassware, material for closure; storage; packaging, labeling; the order of actions in emergencies
Quality control	The procedure for the in-house quality control; weighing on an analytical balance; potentiometric determination of pH[8]; acid-base titration using the colour indicator solution, etc.
Carrying out works under the contract	Contract signing; repair works; external audit; quality control in an independent laboratory; sampling and transport
Complaints and recall	Storage of the raw material; managing customer complaints, conflict resolution; side effects of CP
Self-inspection	The procedure for self-inspection; the SOP review procedure; compliance with the requirements of SOPs; assessment of the premises and equipment

Ukraine, orders of the Ministry of Public Health, and requirements of the State Pharmacopoeia of Ukraine), the literature (scientific publications, reference books), manuals for equipment and take into account the experience of the personnel involved in performance of this procedure. The SOP content should be unambiguous for understanding; it should not contain false and unnecessary information.

According to the modern recommendations for the SOP development an author must answer six questions: "Who?", "What?", "When?", "Where?", "Why?", "How?" [11]. In order to answer to the question "Who?" it is necessary to write the name of the person who is responsible for performing the procedure itself or for the procedure's control; "What?" – describe briefly the procedure to be performed and the resources required; "When?" – determine the timing of the procedure; "Where?" – give the department's name, premises, in which the procedure should be carried out; "Why?" – give the aim of the procedure; "How?" – describe the order / sequence of performing the task. After receiving the answers to these six questions, the document shall be filled according to the SOP format.

The SOPs developed must be signed by *responsible persons* of the compounding pharmacy and approved by the legal entity or individual entrepreneur. The SOPs should be developed for each workplace depending on the duties performed by employees of the pharmacy. Since the production capabilities of various pharmacies are different, the content of the SOPs can not be the same for all pharmacies. The mandatory list of the SOPs must be designed for each compounding pharmacy. It is advisable to give the content of SOPs in the form of tables and/or algorithms.

SOP approval. The written SOPs must be revised. A staff member who was not involved in writing the SOPs should be appointed to review the SOPs. During the SOPs review some questions should be asked: "Have the following six questions (who, what, when, where, why, how) been answered? Are there any restrictions to use the SOP? Has there been something missed?" After the final review the SOP must be approved and signed specifying the date.

All SOPs developed must be registered in the logbook [4] where the SOP name, the registration number, version, the name (position) of the person who developed the SOPs, the name (position) of the person who approved the SOPs, the number of the SOP copies, the place of storage should be noted.

Revision. It is necessary annually to review the relevance of the SOP content, steps/procedures, equipment, etc. If the data is outdated, SOPs should be updated. The SOPs should be also reviewed by employees who directly perform the appropriate procedure, but not by the responsible person developed this SOP. The revised SOPs must be recorded in the SOP registration logbook.

Today there is no form legally approved for SOPs in Ukraine, therefore, each compounding pharmacy can develop SOPs in its own way. However, a certain format and structure of the SOP should be followed [3, 9]. The mandatory SOPs elements are: the title (name of the institution, the name of the SOP, ID number, the version of the SOP, the date of the SOP approving, the signature of the responsible person); the goal; the scope; the responsibility (who performs and who is responsible for the execution, who is responsible for admission of the staff to perform this procedure, and who has the right to replace the employee to perform the procedure); the stages of the procedure; review (valid date, date of review).

Additional information may include the data about the version of the SOPs (developed first, updated, re-

placement of the old SOPs); reference (in the reference books, regulatory and normative documents, etc.); term definitions; materials and equipment; safety. These data can be distinguished as the individual structural elements [12].

SOP systematization. It is appropriate to use the organizational structure of the quality assurance system for the SOP systematization in compounding pharmacies of Ukraine. This structure is given in the guideline for preparation of medicines in healthcare institutions [13, 14]. It is proposed to develop SOPs for each section. The list of typical SOPs for the quality assurance system in compounding pharmacies is given in Table.

CONCLUSIONS

This article presents the methodology of the SOP development for compounding pharmacies of Ukraine. The algorithm of development, approval and revision procedures of SOPs, the structure and the content of a typical SOP, as well as the systematization system have been proposed.

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РОЗРОБКА СТАНДАРТНИХ ОПЕРАЦІЙНИХ ПРОЦЕДУР ТА ЇХ ВПРОВАДЖЕННЯ У ВИРОБНИЧИХ АПТЕКАХ

О.А.Здорик

Ключові слова: *стандартна операційна процедура; лікарські засоби аптечного виготовлення; забезпечення якості лікарських засобів*

Відповідно до вимог належної виробничої практики належне ведення документації у аптеках, що мають ліцензію на виготовлення лікарських засобів, має становити невід'ємну частину системи забезпечення якості та бути ключовим елементом на всіх стадіях виготовлення та контролю якості екстемпоральних лікарських засобів. Одним з важливих елементів належного ведення документації є практика використання стандартних операційних процедур (СОП). Метою даної роботи є виклад методології розробки СОП для аптек, що мають ліцензію на виготовлення лікарських засобів. Стандартні процедури мають розроблятися з урахуванням сучасної нормативної бази та наукових досліджень персоналом, який залучений до виконання даної процедури. СОП мають бути прорецензовані відповідальними особами та затверджені керівником. Виділені наступні структурні елементи СОП: титул (назва установи, назва СОП, класифікаційний номер, версія СОП, дата, з якої СОП набувають чинності, підпис відповідальної особи); мета; сфера застосування; відповідальність; стадії процедури;

перегляд (термін дії, коли СОП мають бути переглянуті). Для систематизації СОП запропоновано використовувати організаційну структуру системи забезпечення якості: забезпечення якості, персонал, приміщення та обладнання, документація, технологія виготовлення, контроль якості, проведення робіт за контрактом, скарги та відкликання, самоінспекція. Для кожного з розділів пропонується розробляти СОП, орієнтовані на забезпечення виконання тієї процедури і забезпечення того аспекту якості, якому присвячено цей розділ.

РАЗРАБОТКА СТАНДАРТНЫХ ОПЕРАЦИОННЫХ ПРОЦЕДУР И ИХ ВНЕДРЕНИЕ В ПРОИЗВОДСТВЕННЫХ АПТЕКАХ

А.А.Здорик

Ключевые слова: *стандартная операционная процедура; лекарственные средства аптечного приготовления; обеспечение качества лекарственных средств*

В соответствии с требованиями надлежащей производственной практики, надлежащее ведение документации в аптеках, имеющих лицензию на приготовление лекарственных средств, должно составлять неотъемлемую часть системы обеспечения качества и быть ключевым элементом на всех стадиях приготовления и контроля качества экстемпоральных лекарственных средств. Одним из важных элементов надлежащего ведения документации является практика использования стандартных операционных процедур (СОП). Целью данной работы является изложение методологии разработки СОП для аптек, имеющих лицензию на приготовление лекарственных средств. Стандартные процедуры должны разрабатываться с учетом современной нормативной базы и научных исследований персоналом, который вовлечен в выполнение данной процедуры. СОП должны быть прорецензированы ответственными лицами и утверждены руководителем. Выделены следующие структурные элементы СОП: титульная страница (название учреждения, название СОП, классификационный номер, версия СОП, дата, с которой СОП вступает в силу, подпись ответственного лица); цель; сфера применения; ответственность; стадии процедуры; пересмотр (срок действия, когда СОП должны быть пересмотрены). Для систематизации СОП предложено использовать организационную структуру системы обеспечения качества: обеспечение качества, персонал, помещение и оборудование, документация, технология приготовления, контроль качества, проведение работ по контракту, жалобы и отзывы, самоинспекция. Для каждого из разделов предлагается разрабатывать СОП, ориентированные на обеспечение выполнения той процедуры и обеспечение того аспекта качества, которому посвящен этот раздел.

Recommended by Doctor of Pharmacy, professor N.P.Polovko

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THE STUDY OF CONSUMER ASPECTS OF MEDICAL PRODUCTS FOR DIAGNOSIS OF PREGNANCY

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Key words: human chorionic gonadotropin; pregnancy test; consumer aspects; pregnancy diagnosing

To diagnose pregnancy means to ascertain the fact of pregnancy and its duration. Timely and accurate diagnosis of pregnancy and determination of its term are needed to prevent complications, premature birth and prolongation. There are several kinds of tests for pregnancy at the market such as conventional test strips, tablets, midstream sticks and electronic tests. Medical products for diagnosis of pregnancy are widely popular among women because of their high efficiency and reliability of the results. The leaders among manufacturing countries are Canada, Germany and the USA. It has been found that there are tests with different sensitivity of 30, 25, 20 and 10 Mme/ml. The least sensitive are simple strips (20-15 mIU/ml), and midstream sticks have the highest sensitivity (10 mIU/mL). The analysis of sensitivity indicators of tests has been conducted using of the standards WHO IS 75/537 and IRP 75/537. It has been found that such tests as Sezam, HomeTest and ULTRA, which can be used on the 7th day after fertilization, have the highest sensitivity.

According to the Ministry of Public Health, Ukraine ranks the fifth place by population among the European countries and the second place among the CIS countries. But, unfortunately, a deplorable fact is that for over 20 years in Ukraine the annual number of deaths exceeds the number of births [4]. Therefore, there is an urgent need for early diagnosis of pregnancy; it allows to follow specific recommendations for its preservation, appropriate correction of the life plans of future parents; diet, work, sexual activity and other aspects of the life of a pregnant woman.

To diagnose pregnancy means to ascertain the fact of pregnancy and its duration. Timely and accurate diagnosis of pregnancy and determination of its term are needed to prevent complications, premature birth and prolongation. In addition, early diagnosis of pregnancy can eliminate the influence of harmful factors in the first weeks of pregnancy [7].

Today, there is a number of methods for diagnosis of pregnancy used both in hospitals and at home. These methods differ in a number of features, higher or lower specificity and reliability. Pregnancy tests are the most widely used.

A pregnancy test is a rapid qualitative immunochromatographic test to determine human chorionic gonadotropin (hCG) in urine samples [5, 6]. Human chorionic gonadotropin is a glycoprotein hormone produced from the first hours of pregnancy [6, 9]. The analysis of the literature data shows that in the normal course of pregnancy hCG is found in the urine soon after conception and reaches the level of 5-50 mIU/ml during the first week of pregnancy [8, 11]. Over the next 10 weeks the level of its concentration increases rapidly and reaches 100000-200000 mIU/ml at the end of the first trimester. The presence of hCG in the urine soon after conception and the rapid increase in its concentration is an indicator of pregnancy [1, 2]. It is known that in women with early terms of pregnancy the hCG level is approximately the same, and it is this fact that makes it an ideal marker for quick and accurate determination of pregnancy [9, 10].

There are several kinds of tests for pregnancy at the market such as conventional test strips, tablets, midstream sticks and electronic tests.

Test strips are required to be immersed in a container with the urine, so some inconvenience of their use is the need to use a separate container for collection of the urine, as well as there is the probability of an erroneous test result.

Tablet tests are a plastic box with two holes. In one of them a few drops of urine are put (for this purpose a pack contains a pipette), the second hole is for observing results. Their drawback is also the need for additional container for collection of the urine into the pipette.

Midstream sticks of the third generation are more convenient and accurate. For testing there is no need to collect urine, in addition, they can be used at any time of the day [3].

Electronic tests, which allow to get a definite answer about the presence or absence of pregnancy, work on the principle of previous products, but have a much higher price. Application of the latest technologies has allowed to provide a woman with such modern product as reusable pregnancy test. It provides almost one hundred percent accuracy, can be reused (up to 20 times) and has the additional function of calculating the probable date of birth.

The aim of the work was to study the range and indices of consumer properties of pregnancy tests presented at the pharmaceutical market of Ukraine.

Materials and Methods

The work analyzes the market of medical products for diagnosis of pregnancy presented by many domestic and foreign manufacturers. Dependence of sensitivity of tests on the term of the possible pregnancy determination has been also found.

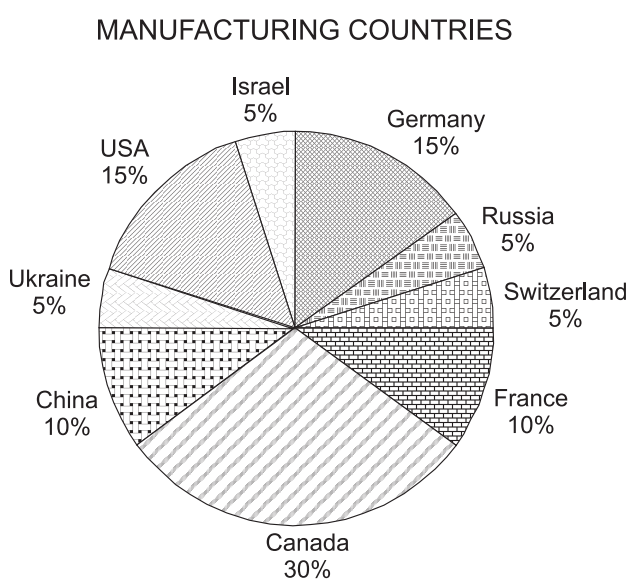

Fig. 1. Manufacturing countries of products for pregnancy diagnosing presented at the Ukrainian market.

Results and Discussion

Medical products for diagnosis of pregnancy are widely popular among women because of their high efficiency and reliability of the results. The leaders among manufacturing countries are Canada, Germany and the USA (Fig. 1, Tab. 1) [2, 3].

One of the factors when choosing a pregnancy test is its sensitivity, which is usually indicated on the pack or in the package insert for the product. The lower concentration of the hormone in the urine the test is able to recognize, the more sensitive it is. It has been found that there are tests with different sensitivity of 30, 25, 20 and 10 Mme/ml. The least sensitive are simple strips (20-15 mIU/ml), and midstream sticks have the highest sensitivity (10 mIU/mL).We have also clarified the relationship between sensitivity of tests and the pregnancy term in which they can be used (Tab. 2).

The analysis of sensitivity indicators of tests has been conducted using of the standards WHO IS 75/537 and IRP 75/537. It has been found that such tests as Sezam,

Table 1

The range of pregnancy tests presented at the Ukrainian market

Trade name	Manufacturer	Country of origin
Frautest	Human Gesellschaft	Germany
Evitest	Helm	Germany
Be sure	Progressive BIO MED Technologies	Russia
Test FOR Best	Schonen	Switzerland
BB Test	Innotech International	France
Cameo	Pharmascience	Canada
Miss	World of Health Biotech	China
Bona	Tespro	Ukraine
Be sure	Ind diagnostic Inc.	Canada
Clear Girl	ZER HITECH	Israel
Babychek	VEDA-LAB	France
Evidence	LEB-International	USA
Answer	LEB-International,	USA
My SECRET	Ind diagnostic Inc. ,	Canada
My SECRET-Lux	Ind diagnostic Inc.	Canada
Nouwelle	Ind diagnostic Inc.	Canada
Secret	ACON LABS	USA
Duet	IND diagnostic Inc.	Canada
Ultra	ACON BIOTECH	China
ITEST	AXIOM	Germany

Table 2

Dependence of sensitivity of the tests on the term of pregnancy

Sensitivity of the test, mIU/ml	The possibility of determining pregnancy
10 mIU/ml	On the 7th day after fertilization
20 mIU/ml	On the 7-10th day after fertilization
25 mIU/ml	From the 1st day after the missed period

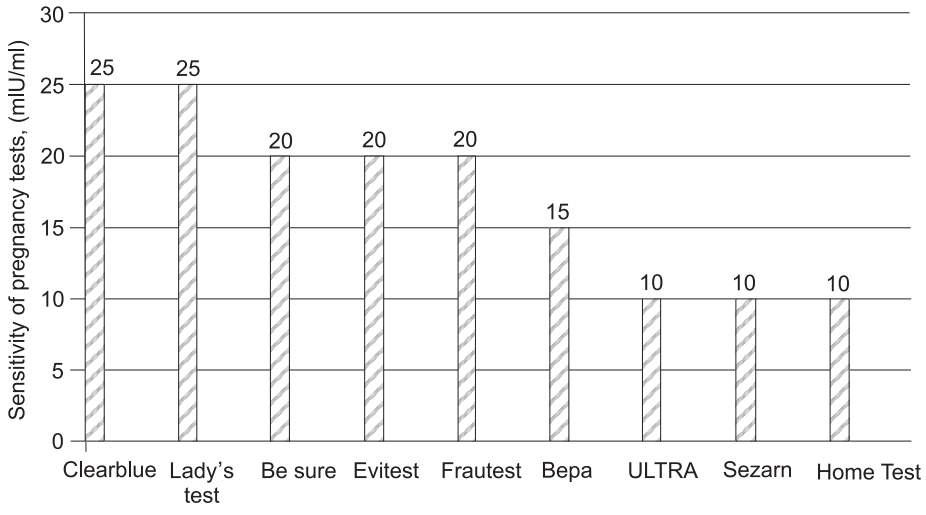

Fig. 2. Sensitivity of pregnancy tests.

HomeTest and ULTRA, which can be used on the 7th day after fertilization, have the highest sensitivity (Fig. 2).

Based on the data from the literature sources it has been found that pregnancy tests can be stored at low (refrigerator) and room temperature (between 2°C to 30°C). Their stability preserves until the expiration date (24 months from the date of manufacture). These data indicate that the given products do not require special storage conditions, and it is a positive consumer characteristic.

CONCLUSIONS

The analysis of the pharmaceutical market of Ukraine where medical products in the form of pregnancy tests are presented has shown that the leaders among manufacturing countries are Canada, Germany and the USA.

The analysis of sensitivity indicators of pregnancy tests has revealed that such tests as Sezam, HomeTest and ULTRA, which can be used on the 7th day after fertilization, have the highest sensitivity.

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ДОСЛІДЖЕННЯ СПОЖИВЧИХ АСПЕКТІВ ВИРОБІВ МЕДИЧНОГО ПРИЗНАЧЕННЯ ДЛЯ ДІАГНОСТИКИ ВАГІТНОСТІ

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Ключові слова: *хоріонічний гонадотропін; тест на вагітність; споживчі аспекти; діагностування вагітності*

Діагностувати вагітність – означає встановити факт вагітності та її термін. Своєчасний і точний діагноз вагітності та визначення її терміну необхідні для профілактики ускладнень, передчасних пологів і переношування. На ринку представлені декілька видів тестів на вагітність: звичайні тест-смужки, планшетні, струминні та електронні тести. Вироби медичного призначення для діагностики вагітності користуються широкою популярністю серед жінок через свою високу ефективність та достовірність результатів. Лідерами серед країн-виробників є Канада, Німеччина та США. Встановлено, що пропонуються тести з різною чутливістю: 30, 25, 20 і 10 Mme/ml. Найменш чутливими є прості стрип-смуги (20-15 мМО/мл), а найбільшу чутливість мають струминні тести (10 мМО/мл). Аналіз показника чутливості тестів проводився з використанням стандартів ВООЗ IS 75/537 та IRP 75/537. Встановлено, що найбільшу чутливість мають тести Sezam, HomeTest і ULTRA, які можна використовувати на 7-й день після запліднення.

ИССЛЕДОВАНИЕ ПОТРЕБИТЕЛЬСКИХ АСПЕКТОВ ИЗДЕЛИЙ МЕДИЦИНСКОГО НАЗНАЧЕНИЯ ДЛЯ ДИАГНОСТИКИ БЕРЕМЕННОСТИ

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Ключевые слова: *хорионический гонадотропин; тест на беременность; потребительские аспекты; диагностирование беременности*

Диагностировать беременность – значит установить факт беременности и ее срок. Своевременный и точный диагноз беременности и определение ее срока необходимы для профилактики осложнений, преждевременных родов и перенашивания. На рынке представлено несколько видов тестов на беременность: обычные тест-полоски, планшетные, струйные и электронные тесты. Изделия медицинского назначения для диагностики беременности пользуются широкой популярностью среди женщин благодаря своей высокой эффективности и достоверности результатов. Лидерами среди стран-производителей являются Канада, Германия и США. Установлено, что предлагаются тесты с разной чувствительностью: 30, 25, 20 и 10 Mme/ml. Наименее чувствительными являются простые стрип-полоски (20-15 мМО/мл), а наибольшую чувствительность имеют струйные тесты (10 мМО/мл). Анализ показателя чувствительности тестов проводился с использованием стандартов ВОЗ IS 75/537 и IRP 75/537. Установлено, что наибольшую чувствительность имеют тесты Sezam, HomeTest и ULTRA, которые можно использовать на 7-й день после оплодотворения.

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ANALYSIS OF THE DOMESTIC MARKET OF PLANT-BASED MEDICINES

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Key words: pharmaceutical market; analysis; medicines; herbal remedies

Therapy with medicinal plants and remedies on their basis from time immemorial has been used by mankind to treat a variety of diseases and has not lost its relevance up to now. Today, phytomedicines are used both in the complex therapy (as a complementary element) and independently. There are medicines of the plant origin and herbal remedies. The aim of this study was to analyse the range of phytomedicines registered at the pharmaceutical market of Ukraine. In the section "Herbal medicines" of Reference book of medicines of Ukraine (04/01/2013) 992 names of medicines are presented, except of substances and medicines in bulk. The study of this range has shown that the vast majority of medicines are manufactured in Ukraine. Medicines produced in Germany and India prevail among foreign phytomedicines. Subsequent analysis of dosage forms of phytomedicines has revealed that the largest number of medicines comes to the domestic market in the form of the medicinal plant raw material, tablets, tinctures, capsules, solutions and syrups. Distribution of phytomedicines by the age limits has been studied. It has been found that the most of medicines are intended for use by children above 12 years old and adults. Thus, it has been found that there are only few phytomedicines for younger children, whereas phytotherapy is the most appropriate for treating children of different age groups. This fact makes developing of new domestic phytomedicines for use in pediatrics particularly relevant and reasonable.

Herbal medicines (hereinafter – phytomedicines) occupy a significant place among drugs of conventional medicine, and their use is important due to a number of advantages in comparison with medicines of the synthetic origin. Thus, they are more environmentally friendly, have a polyvalent effect, at acceptable prices, their use may be long-term, etc. [1, 4, 5, 7, 8].

Nowadays phytomedicines are actively used in the complex therapy as a complementary element that supplements and enhances the therapeutic effect of treatment [6, 9, 10].

Phytomedicines include medicines of the plant origin ("any finished medicinal product containing active substances from one or more plant substances, or active substances from one or more plant substances in combination with other medicinal products") and herbal remedies ("medicines obtained from processing of plant substances by extraction, distillation, pressing, fractionation, purification, concentration and fermentation. They include crushed or powdered plant substances, tinctures, extracts, essential oils, expressed juices and processed exudates") [3].

For the purpose of analysis [2] of the domestic market of phytomedicines they have been studied by the place of manufacturing, distribution by the type of a dosage form and age limits.

Materials and Methods

The range of phytomedicines registered at the Reference book of medicines of Ukraine (04/01/2013) was studied by manufacturing countries (Fig. 1, Tab. 1).

The analysis of nomenclature by the type of a dosage form (Tab. 2) was conducted, and distribution of phytomedicines by the age category (Fig. 2) was studied.

Results and Discussion

The Reference book of medicines of Ukraine (04/01/2013), section "Herbal medicines", includes 992 medicines, excluding substances and medicines *in bulk* [3].

It has been found that in the range selected phytomedicines of domestic production prevail – there are 651 names registered at the market (Fig. 1).

Taking into account that the share of phytomedicines of foreign production is significant, their structure by manufacturing country was studied. It has been found that suppliers of phytomedicines to the domestic market are manufacturers from 32 countries (Tab. 1).

It is seen from Table 1 that suppliers of phytomedicines from Germany (22.91%), India (14.65%), Pakistan (7.61%), Poland (7.03%) and Slovenia (6.73%) dominate at the domestic pharmaceutical market.

The next stage of the study was analysis of the range of medicines selected by the type of a dosage form, in which they enter the market since the therapeutic effect of the medicine largely depends exactly on the dosage form. The type of the dosage form is also important in the individual approach to treatment of each patient and in treating diseases of varying severity.

It has been found that phytomedicines are presented as 27 dosage forms at the domestic pharmaceutical market (Tab. 2).

Table 2 demonstrates that the medicinal plant raw material (16.63%), tablets (14.31%), tinctures (12.50%), capsules (9.68%), solutions (6.96%) and syrups (6.55%) predominate.

The vast majority of medicines in the form of the medicinal plant raw material are medicines of domestic

Table 1

The structure of phytomedicines of foreign production at the domestic pharmaceutical market by manufacturing countries

Manufacturing countries	The share of phytomedicines at the domestic pharmaceutical market, %
Germany	22.91
India	14.65
Pakistan	7.61
Poland	7.03
Slovenia	6.73
Vietnam	4.40
Czech Republic	4.10
France	4.10
The Russian Federation	3.22
Austria	2.63
Australia	2.63
Bulgaria	2.05
Hungary	1.76
USA	1.76
Netherlands	1.76
Great Britain	1.47
China	1.17
Slovak Republic	1.17
Georgia	0.88
The Republic of Belarus	0.88
Thailand	0.88
Switzerland	0.88
Turkey	0.56
Romania	0.56
Egypt	0.56
Denmark	0.56
Moldova	0.56
Canada	0.56
Spain	0.56
Bosnia and Herzegovina	0.56
Estonia	0.56
Sri Lanka	0.29
Total	100.0

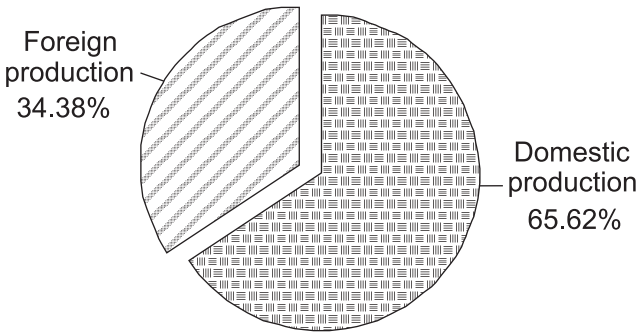


Fig. 1. The origin of phytomedicines registered at the Ukrainian pharmaceutical market by the place of production.

Table 2

Distribution of phytomedicines at the domestic pharmaceutical market by the dosage forms

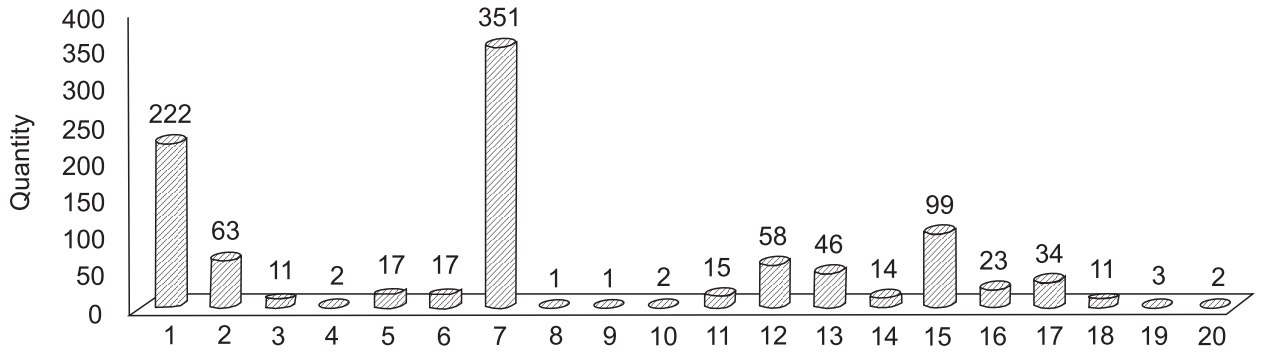
Dosage form	The number of phytomedicines in this dosage form	The share at the pharmaceutical market of Ukraine, %
Medicinal plant raw material	165	16.63
Tablets	142	14.31
Tincture	124	12.50
Capsules	96	9.68
Solution	69	6.96
Syrup	65	6.55
Drops	47	4.74
Herbal tea	43	4.33
Ointment	37	3.73
Extract	21	2.12
Pastilles	21	2.12
Oil	19	1.92
Lozenges (candies)	18	1.81
Balm	17	1.71
Granules	16	1.61
Gel	16	1.61
Suppositories	13	1.31
Spray	13	1.31
Fluid	12	1.21
Powder	12	1.21
Tea	11	1.11
Liniment	6	0.60
Shampoo	3	0.31
Elixir	3	0.31
Juice	1	0.10
Dragee	1	0.10
Pills	1	0.10
Total	992	100.0

production, while many of them do not have additional packaging to filter bags, but it is inconvenient for their further use by patients.

Very few phytomedicines are represented in such dosage forms as juices, dragee and pills (0.10% each) at the domestic pharmaceutical market. In our opinion, when creating new medicines based on the plant material it is advisable to consider this fact when choosing the dosage form.

Phytomedicines provide a “soft” therapeutic effect at a low toxicity, cause significantly fewer side effects and are usually much easier tolerated by patients compared to synthetic medicines. Therefore, phytomedicines form the basic range of medicines of choice in pediatrics.

The further analysis of the age limits for using phytomedicines has shown that they are divided into 20 groups (Fig. 2). The vast majority of medicines (351 names) are provided for the use of children above 12 years and



1 – Not applicable in pediatrics	6 – Above 14 years	11 – Above 7 years	16 – Above 2 years
2 – Applicable	7 – Above 12 years	12 – Above 6 years	17 – Above 1 year
3 – Above 18 years	8 – Above 10 years	13 – Above 5 years	18 – Above 6 months
4 – Above 16 years	9 – Above 9 years	14 – Above 4 years	19 – Above 3 months
5 – Above 15 years	10 – Above 8 years	15 – Above 3 years	20 – Above 28 days

Fig. 2. The age limits specified in patient information leaflets for phytomedicines that are presented at the domestic pharmaceutical market.

adults. The second largest group of phytomedicines (222 names) is those medicines that are not used in pediatrics. Of 992 phytomedicines registered only 99 of them can be used to treat children aged above 3 years. Among phytomedicines that can be used without the age limit (63 names) the significant part takes those that do not contain individually specified information and recommendations for their use by children, causing some doubts about the possibility of their use by this age group.

It is important to note that some domestic phytomedicines in the form of the medicinal plant raw material, which are analogues in the composition and a dosage form but produced by different companies, have conflicting data regarding their safety for children. For example, the patient information leaflet of "Linden flowers" manufactured by KP Kyiv Regional Council "Pharmaceutical Factory" (Kyiv) indicates that "when applying the medicine it should be considered that the safety and efficiency of its use in the treatment of children under 12 years old have not been found, that is why the medicine for patients in this age group should be used with caution". Herewith, "Linden flowers" produced by "Liktravy" PJSC (Zhitomir) and Pharmaceutical factory "Viola" PJSC (Zaporizhzhya) specify an age limit as – for children above 3 years. Such differences may cause difficulties for patients.

CONCLUSIONS

1. The range of phytomedicines registered in the Reference book of medicines of Ukraine (04/01/2013), section "Herbal medicines" has been studied. It has been found that the vast majority of medicines are produced in Ukraine (65.62%). Manufactures from Germany (22.91%) and India (14.65%) prevail among foreign suppliers.

2. The analysis of the range of medicines selected has revealed that the largest number of medicines comes to the domestic market in the form of the medicinal plant raw material (16.63%), tablets (14.31%), tinctures (12.50%), capsules (9,68%), solutions (6.96%) and syrups (6.55%).

3. Distribution of phytomedicines by the age limits has been studied. The most of medicines are intended for use by children above 12 years old (35.38%) and adults (22.38%).

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АНАЛІЗ ВІТЧИЗНЯНОГО РИНКУ ЛІКАРСЬКИХ ПРЕПАРАТІВ НА РОСЛИННІЙ ОСНОВІ

К.В.Толочко

Ключові слова: *фармацевтичний ринок; аналіз; лікарські препарати; рослинні засоби*

Терапія лікарськими рослинами і засобами на їх основі споконвіку використовувалася людством для лікування різноманітних захворювань і до цього дня не втратила своєї актуальності. На сьогоднішній день фітопрепарати активно застосовуються як у складі комплексної терапії (як комплементарний елемент), так і самостійно. Виділяють лікарські препарати рослинного походження і рослинні лікарські засоби. Метою даної роботи став аналіз асортименту фітопрепаратів, зареєстрованих на фармацевтичному ринку України. У розділі «Рослинні препарати» Довідника лікарських засобів України (01.04.2013) представлено 992 лікарських препарати крім субстанцій та препаратів in bulk. Дослідження виділеного асортименту показало, що переважна більшість препаратів виготовляється в Україні. Серед зарубіжних фітопрепаратів переважають лікарські засоби виробництва Німеччини та Індії. Подальший аналіз лікарських форм фітопрепаратів дозволив встановити, що найбільша кількість препаратів надходить на вітчизняний ринок у вигляді лікарської рослинної сировини, таблеток, настойок, капсул, розчинів та сиропів. Вивчено розподіл фітопрепаратів за віковим показником. Встановлено, що більшість препаратів розрахована для вживання дітьми старше 12 років і дорослими. Таким чином, було встановлено, що фітопрепаратів для дітей молодшого віку дуже мало, тоді як саме фітотерапія є найбільш придатною для лікування дітей різних вікових груп. Цей факт обумовлює особливу актуальність і доцільність розробки нових вітчизняних фітопрепаратів для застосування в педіатричній практиці.

АНАЛИЗ ОТЕЧЕСТВЕННОГО РЫНКА ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ НА РАСТИТЕЛЬНОЙ ОСНОВЕ

Е.В.Толочко

Ключевые слова: *фармацевтический рынок; анализ; лекарственные препараты; растительные средства*

Терапия лекарственными растениями и средствами на их основе испокон веков использовалась человечеством для лечения разнообразных заболеваний и по сей день не потеряла своей актуальности. На сегодняшний день фитопрепараты активно применяются как в составе комплексной терапии (как комплементарный элемент), так и самостоятельно. Выделяют лекарственные препараты растительного происхождения и растительные лекарственные средства. Целью данной работы стал анализ ассортимента фитопрепаратов, зарегистрированных на фармацевтическом рынке Украины. В разделе «Растительные препараты» Справочника лекарственных средств Украины (01.04.2013) представлено 992 лекарственных препарата помимо субстанций и препаратов in bulk. Исследование выделенного ассортимента показало, что подавляющее большинство препаратов изготовляется в Украине. Среди зарубежных фитопрепаратов преобладают лекарственные средства производства Германии и Индии. Последующий анализ лекарственных форм фитопрепаратов позволил установить, что наибольшее количество препаратов поступает на отечественный рынок в виде лекарственного растительного сырья, таблеток, настоек, капсул, растворов и сиропов. Изучено распределение фитопрепаратов по возрастному показателю. Установлено, что большинство препаратов рассчитано для употребления детьми старше 12 лет и взрослыми. Таким образом, было установлено, что фитопрепаратов для детей младшего возраста очень мало, тогда как именно фитотерапия является наиболее подходящей для лечения детей разных возрастных групп. Этот факт обусловливает особую актуальность и целесообразность разработки новых отечественных фитопрепаратов для применения в педиатрической практике.

ЕКСПЕРИМЕНТАЛЬНА ТА КЛІНІЧНА ФАРМАКОЛОГІЯ

Recommended by Doctor of Pharmacy, professor N.V.Khokhlenkova

UDC 615.015.32:616-08:616.211-002

MODERN ASPECTS OF DEVELOPMENT AND TREATMENT OF RHINOSINUSITIS

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Key words: chronic diseases; rhinosinusitis; therapy; drugs; homeopathic medicines

Rhinosinusitis is one of the most common chronic diseases. The studies in recent decades have shown that approximately 10% of rhinosinusitis have an endogenous nature. According to statistics, women more often suffer from rhinosinusitis than men, as well as people from the higher social strata of society. According to the literature, patients with rhinosinusitis are about one third of the total number of hospitalizations in ENT in-patient departments. Foci of inflammation in the paranasal sinuses can be a source of infectious sensitization of the respiratory tract and lungs, as well as the cause of severe intracranial complications. Therefore, the problem of the treatment of rhinosinusitis remains one of the most urgent and difficult in otorhinolaryngology. The empirical antibiotic therapy is the basic treatment of acute bacterial rhinosinusitis, as well as exacerbation of chronic rhinosinusitis. Drugs of the first choice in acute rhinosinusitis are amoxicillin, amoxicillin/clavulanate. Cephalosporins, e.g., cefuroxime axetil, are another way of treatment. Drugs, which are prescribed in the case of failure of the first course of antibiotics, are macrolides and fluoroquinolones of the III-IV generations such as levofloxacin and moxifloxacin. The use of traditional medicines has a positive therapeutic effect, but side effects of glucocorticoids and vasoconstrictors for topical application are associated with the risk of retinal vascular embolism and development blindness. Therefore, today the use of homeopathic complex drugs based on the components of the plant, animal and mineral origin is topical since they are as effective as allopathic drugs, do not exhibit undesirable side effects, drug intolerance and the effect of habituation, and do not cause allergic reactions.

Nowadays there is the increased progression of nasal phlogistic diseases, especially in chronical forms, it often leads to the loss of labour capacity, and if the intracranial complications are developed, it can result in patient's disability and death. According to the WHO data 235 million people suffer from asthma, 64 million have lungs chronic obstructions, while billions of people are diagnosed with allergic rhinitis and other chronic respiratory diseases [4, 5].

The nasal cavity and sinus are the highly organized structure with subtle and complicated regulation mechanisms possessing a lot of specific functions.

Rhinosinusitis is inflammation of the sinus and nasal cavity caused by congestion of secretion and aeration disorder. The starting point for development of rhinosinusitis is an acute respiratory viral infection [13].

According to the American Healthcare Bureau 14.7% of the Americans suffer from rhinosinusites. According to the US Disease Statistics Center rhinosinusitis has become the most spread chronic disease in the country, exceeding the indicators of the diagnosis of arthritis and hypertension. Dependence of the disease on the sex of the patients, the place of residence and the social status has been also studied. According to the US statistics, women are suffering from rhinosinusitis more often than men, as well as people from the higher social strata of society [9, 11].

In addition, studies conducted in Russia have proven that patients with rhinosinusitis have significantly lower indices of pain sensitivity and social activity than patients with coronary heart disease and chronic obstructive pulmonary disease. In 26% of patients rhinosinusitis is accompanied by development or progression of mental depression [1].

When studying the structure of morbidity of the otolaryngology organs in Moscow city clinical hospital No.4 it has been found that the largest nosological group includes diseases of the nasal cavity and paranasal sinuses (~54%, Fig.). The ear pathology (25%) is the second, and diseases of the pharynx (10%) are the third [1].

The analysis of the statistical data shows that there is a consistently high percentage of prevalence of diseases of the nasal cavity and paranasal sinuses. Due to that it is vital to develop the measures to improve the medical care of patients with these diseases [1, 12].

In recent years the incidence of rhinosinusitis has almost 3 times increased, while the number of hospitalized people has increased by 1.5-2%. Thus, the problem of treatment of rhinosinusitis remains one of the most urgent and complex [4].

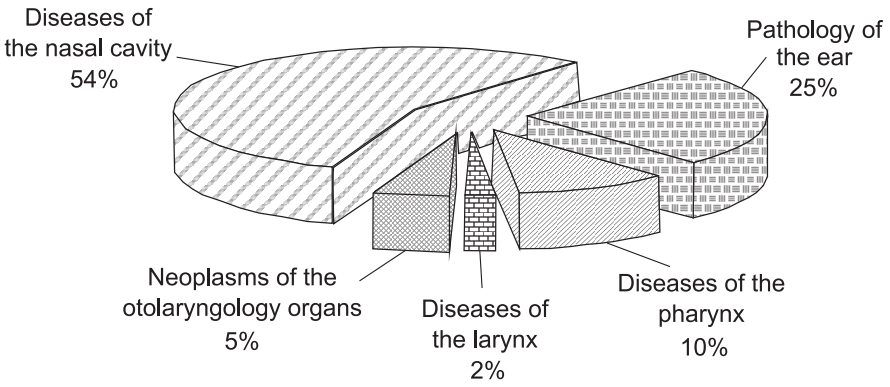

Fig. The structure of morbidity of the otolaryngology organs.

The main aim of treatment of rhinosinusitis is to reduce duration of the disease; prevent the intracranial complications; eradicate the pathogen. In that way the basic method of treatment of acute bacterial rhinosinusitis and exacerbation of chronic rhinosinusitis is an empiric antibacterial therapy (amoxicillin/clavulanate, etc.) [2].

In the USA the frequency of using antimicrobial agents reaches 80%, in Europe – 72-92% [13].

The disease ranks 5th among diseases when antibiotics are prescribed: the USA spends approximately 6 billion $ to buy antibiotics for treating rhinosinusitis [14].

According to the pharmacoepidemiological research conducted in Russia it has been found that amoxicillin is a drug of the first choice being prescribed to 18% of patients. In 13.5% of cases ciprophloxacin is prescribed, ampicillin – in 12% of cases, doxycyclin – in 7.7% of cases and stand for gentamicin – in 5.5% of cases. Amoxicillin is prescribed only for 16.7% of patients, amoxicillin/clavulanate – only for 3.1% of patients [7, 9].

However, in practice, there are often situations when the choice of treatment of rhinosinusitis is complicated by intolerance to antibiotics.

Such vasoconstrictors as naphazoline, oxymetazoline, xylometazoline become essential in therapy of rhinosinusitis; they affect the nasal cavity tonus regulation. But the use of these medicines can cause edema in the nasal cavity, changes in histological structure of the mucosa, i.e. development of drug-induced rhinitis.

The therapy of acute rhinosinusitis also uses herbal medicines with the antiphlogistic and mucolytic effect (Erespal, Sinuforte, Sinupret). It is the combination of synergistic active substances that provides the complex secretolytic, anti-edema, bronchodilatatory, anti-inflammatory and immunostimulating effects when treating rhinosinusitis [2].

In 2012 based on the results of several randomized controlled clinical studies the updated European recommendations for treatment of rhinosinusitis (European position paper on rhinosinusitis and nasal polyps (EPOS)) were published. The document reflects the issues of determination, including the classification and epidemiology, contributing to the factors of development and treatment of rhinosinusitis. One of the new aspects of EPOS-2012 is the inclusion of recommendations for the treatment of some forms of rhinosinusitis with herbal drugs (recommendations A) [11].

Tab. 1 generalizes the recommendations on the therapy of acute rhinosinusitis in adults containing in the EPOS up-to-date edition (2012).

The herbal drugs possess many advantages compared to the synthetic ones, e.g. when using medicines from plants allergic reactions and unwanted effects develop less often [6].

Combining the methods of traditional medicine and homeopathy allows to achieve a relatively quick desired effect when treating many diseases. The efficiency, safety, the absence of side effects, economic availability make homeopathic medicines indispensable for use in children, pregnant women, elder people and patients with a tendency to allergic reactions [8].

The search of new medicines by studying the toxic and dynamic properties (“medicinal pathogenesis” ac-

Table 1

The recommendation level when treating acute rhinosinusitis (EPOS-2012)

Therapy	Recommendation level	Indication
Antibiotics	A	In the case of acute bacterial rhinosinusitis
Topical (intranasal) HCSs	A	In the case of allergic rhinosinusitis
Saline nose washing	B	In the case of all rhinosinusitis forms
Anti-histamines + decongestants	A	In the case of acute viral rhinosinusitis
Probiotics	A	For prophylaxis of acute rhinosinusitis
Phytodrugs, aspirin, paracetamol	A	In the case of acute viral rhinosinusitis
Decongestants, mucolytics	D	Not used
Zinc, vitamin C, Echinacea	C	Not used
Steam inhalations	A	Not used (ineffective)

Table 2

Complex homeopathic medicines used in the therapy of rhinosinusitis

Name	Manufacturer	Dosage form	Indications
Cinnabsin	Deutsche Homeopathy-Union DHU (Germany)	Homeopathic tablets	Complex therapy of acute and chronic nasal inflammations (sinusitis, frontal sinusitis)
Rhinital	DHU (Germany)	Homeopathic tablets	Allergic rhinitis as a part of complex therapy
Asinis	Richard Bittner (Austria)	Homeopathic drops	Complex therapy of sinusitis, frontal sinusitis and chronic rhinitis
Euphobium Compositum Nasentropfen C	Heel (Germany)	Homeopathic nasal spray	Rhinitis of different etiology, chronic sinusitis
Delufen	Richard Bittner (Austria)	Homeopathic nasal spray	Rhinitis of different etiology, chronic sinusitis, pharyngitis
Edas-131	EDAS (Russia)	Nasal drops	Acute and chronic rhinitis (including allergic one)
Allergie	Walsh Pharma (USA)	Homeopathic tablets	Allergic rhinitis as a part of complex therapy
Coryzalia	Boiron Lab (France)	Homeopathic tablets	Rhinitis (edema and/or irritation of the nasal mucosa, sneezing)

cording to Hannemann) of different organic and inorganic substances is constantly carried out. That is why new homeopathic medicines appear at the pharmaceutical market [10].

The given facts determine the relevance of developing new domestic homeopathic medicines, first of all, their complex forms. The complex homeopathic forms can be considered as the drugs of choice in cases when administration of allopathic medicines are particularly undesirable – during pregnancy, in allergies, among the little children, as well as in diseases of the liver and kidneys. The complex homeopathic medicines have become the essential assortment part in both homeopathic and conventional pharmacies. In the structure of the turnover of large wholesale firms the sale of homeopathic medicines is up to 5 % [3, 8].

The complex homeopathic medicines for prevention and treatment of rhinosinusitis registered in Ukraine are given in Tab. 2.

The assortment presented shows the insufficient quantity of complex homeopathic medicines for rhinosinusitis at the Ukrainian market. The German manufacturers offer 3 drugs for complex therapy of nasal diseases, the Austrian manufacturers – 2 drugs, the Russian, US and French manufacturers – 1 drug per a country for treating rhinitis of different etiology. Besides, there are no domestic medicines. Therefore, it proves the relevance of developing and creating new and effective homeopathic medicines in our country.

CONCLUSIONS

The pharmaceutical, medical and social significance of the problem of rhinosinusitis is conditioned by a high prevalence of this disease, a pronounced decrease in the quality of patients' life, the existence of resistant and recurrent forms.

The main method of drug treatment is antibacterial therapy. Usually the drug of choice for treatment of rhinosinusitis is amoxicillin or amoxicillin clavulanate. The traditional drugs often become ineffective and have different side effects causing complications of the disease.

The combination of antibacterial and homeopathic therapy allows to considerably improving the results of treatment of patients with this pathology. The assortment of complex homeopathic medicines for rhinosinusitis at the Ukrainian market is limited, and there are no domestic drugs.

The further studies on development of the composition and technology of a complex homeopathic medicine for use in practical otolaryngology, in particular for prevention and treatment of rhinosinusitis, are required.

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СУЧАСНІ АСПЕКТИ РОЗВИТКУ ТА ЛІКУВАННЯ РИНОСИНУСИТУ

С.В.Олійник

Ключові слова: *хронічні захворювання; риносинусит; терапія; лікарські препарати; гомеопатичні лікарські засоби*

Риносинусит відноситься до числа найпоширеніших хронічних захворювань. Дослідження останнього десятиліття показали, що приблизно 10% риносинуситів мають ендогенну природу. За статистикою на риносинусити частіше хворіють жінки, а також люди з вищих соціальних верств суспільства. За літературними даними хворі на риносинусити становлять близько 1/3 від загального числа госпіталізованих в ЛОР-стаціонари. Вогнища запалення в навколоносових пазухах можуть бути джерелом інфекційної сенсибілізації дихальних шляхів і легенів, а також причиною важких внутрішньочерепних ускладнень. Тому проблема лікування риносинуситів залишається дотепер однією з актуальних і складних в оториноларингології. Базисним методом лікування гострого бактеріального риносинуситу, а також загострення хронічного риносинуситу є емпірична антибактеріальна терапія. Препаратом першого вибору при гострому риносинуситі є амоксицилін, амоксицилін/клавуланат. Іншим варіантом лікування є цефалоспорини, наприклад, цефуроксиму аксетил. Засобами, які призначають у разі неефективності першого курсу антибіотикотерапії, є макроліди та фторохінолони III-IV поколінь: левофлоксацин, моксифлоксацин. Застосування традиційних лікарських засобів чинить позитивний терапевтичний ефект, проте побічні ефекти глюкокортикостероїдів і судинозвужувальних лікарських препаратів для місцевого застосування пов'язані з ризиком емболії судин сітківки та розвитку сліпоти. Тому сьогодні є актуальним застосування гомеопатичних комплексних препаратів на основі компонентів рослинного, тваринного та мінерального походження, оскільки вони за ефективністю не поступаються алопатичним препаратам, не проявляють небажаної побічної дії, ефектів непереносимості препаратів і звикання та не викликають алергічних реакцій.

СОВРЕМЕННЫЕ АСПЕКТЫ РАЗВИТИЯ И ЛЕЧЕНИЯ РИНОСИНУСИТА

С.В.Олейник

Ключевые слова: *хронические заболевания; риносинусит; терапия; лекарственные препараты; гомеопатические лекарственные средства*

Риносинусит относится к числу самых распространенных хронических заболеваний. Исследования последнего десятилетия показали, что примерно 10% риносинуситов имеют эндогенную природу. По статистике риносинуситом чаще болеют женщины, а также люди из высших социальных слоев общества. По литературным данным больные риносинуситом составляют около 1/3 от общего числа госпитализированных в ЛОР-стационары. Очаги воспаления в околоносовых пазухах могут быть источником инфекционной сенсибилизации дыхательных путей и легких, а также причиной тяжелых внутричерепных осложнений. Поэтому проблема лечения риносинусита остается одной из актуальных и сложных в оториноларингологии. Базисным методом лечения острого бактериального риносинусита, а также обострения хронического риносинусита является эмпирическая антибактериальная терапия. Препаратом первого выбора при остром риносинусите являются амоксициллин, амоксициллин/клавуланат. Другим вариантом лечения являются цефалоспорины, например, цефуроксим аксетил. Средствами, которые назначают в случае неэффективности первого курса антибиотикотерапии, являются макролиды и фторхинолоны III-IV поколений: левофлоксацин, моксифлоксацин. Применение традиционных лекарственных средств имеет положительный терапевтический эффект, однако побочные эффекты глюкокортикостероидов и сосудосуживающих лекарственных препаратов для местного применения связаны с риском эмболии сосудов сетчатки и развития слепоты. Поэтому сегодня является актуальным применение гомеопатических комплексных препаратов на основе компонентов растительного, животного и минерального происхождения, поскольку они по эффективности не уступают аллопатическим препаратам, не проявляют нежелательных побочных действий, непереносимости препаратов и эффекта привыкания и не вызывают аллергических реакций.

Recommended by Doctor of Medicine, professor S.Yu.Shtrygol'

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THE TOPICAL ISSUES OF CLINICAL PHARMACOLOGY OF CLARITHROMYCIN

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Key words: clarithromycin; indirect antibacterial effects; nonantibacterial effects; immunomodulatory effect

Recently, as evidenced by numerous publications and surveys on the topic, the interest in clarithromycin is not reduced. This drug has a wide range of the antibacterial activity, is able to penetrate into cells and create stable and high tissue concentration exceeding the level of the drug in the blood serum. However, in recent years, more attention is paid to the study of nonantibacterial effects of clarithromycin. In modern macrolides, in particular in clarithromycin, the anti-inflammatory, immunomodulatory and mucoregulatory properties have been found. The primary mechanism of the immunomodulatory action of macrolides today is considered to be their ability to affect NF-kB-and MAPK-dependent signaling pathways of cells. Under the effect of clarithromycin the decrease in the synthesis and/or secretion of pro-inflammatory (IL-1, -6, -8, tumour necrosis factor (TNF)) and the increase in the secretion of anti-inflammatory cytokines (IL-2, -4, -10) are observed. The effectiveness of the indirect antimicrobial action of clarithromycin is intensively studied in vitro and in vivo, and it further expands our understanding of the therapeutic possibilities of this drug.

Macrolide antibiotics are widely used due to their activity against many pathogens and the presence of a number of therapeutically beneficial nonantibacterial additional properties. Semisynthetic 14-membered macrolide clarithromycin has been successfully used in medical practice for 25 years. The synthesis of clarithromycin was performed by incorporating methoxy at position of the 6-th macrocyclic lactone ring of the erythromycin structure [1, 2, 9].

Clarithromycin inhibits the protein synthesis in bacteria by binding to the active centre of the 50S ribosomal subunit. As a result of the reversible binding and inhibiting the reactions of translocation and transpeptidation, the inhibition of formation and extension of the peptide chain occurs. Recently another ability of macrolides – to disrupt the assembly of the 50S subunit – has been found. The main effect of clarithromycin is bacteriostatic, but at high concentrations and low microbial density relative to *S. pyogenes* and *S. pneumoniae* this drug can exhibit the bactericidal effect [2, 18].

Clarithromycin is active against many gram-positive and gram-negative bacteria, as well as against the majority of intracellular pathogens such as mycoplasma, chlamydia and mycobacteria. It is highly effective against streptococcus, pneumococcus, meningococcus, gonococcus, treponema, clostridium, listeria, corynebacteria diphtheria, anthrax bacillus, *Helicobacter pylori*. The effectiveness of clarithromycin against *Haemophilus influenzae* is due to the antibacterial activity of the basic drug metabolite (14-hydroxy clarithromycin). Many gram-negative bacteria have the natural resistance to macrolides because antibiotics do not penetrate into the cell wall. However, clarithromycin has shown a significant activity *in vitro* and *in vivo* against gram-negative pathogens, such respiratory infections as *Legionella pneumophlia*, *Moraxella catarrhalis*, *Bordetella pertussis*. But macrolides do not affect gram-negative bacteria of such families as *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter*. Clarithromycin is effective against rickettsia, the causative agents of wound infection due to animal bites, active against some anaerobes, including clostridia, bacteroides, actinomycetes, propionibacteria, anaerobic cocci [1, 2, 3, 9].

Acquired resistance of microorganisms to clarithromycin may be due to modification of the target of the antibiotic and active excretion of microbial cells (efflux). Methylation of ribosomes is the main and most studied mechanism of modification of the target. It is characteristic for *Streptococcus spp., Staphylococcus spp*. and is stipulated by the presence of specific genes in these bacteria– erm (erythromycin ribosome methylase) responsible for the synthesis of proteins-methylases; it results in disrupted binding of macrolide with the action target. In this case cross-resistance (the so-called MLSv-type resistance) to macrolides, lincosamides and streptogramins B is formed. Mutations in rRNA and ribosomal proteins L4, L16, L22 are the mechanism of resistance, which clinical significance has not yet been determined. Single mutations in domain V of rRNA are observed in *S. pneumoniae*, *Mycobacterium spp*., *H. pylori*, *B. pertussis*, they lead to the expression of resistance phenotypes MLSv and ML (resistance to macrolides and lincosamides). Mutations in the L-proteins identified in clinical strains of *S. pneumoniae* and *S. pyogenes* cause the resistance to erythromycin while preserving the sensitivity to lincosamides [1, 2, 9, 12].

Active elimination (efflux) of microbial cells is carried out with the proton pump encoded by genes mefA

and mefE [21]. In this case only 14- and 15-membered macrolides, including clarithromycin, are eliminated; the susceptibility to 16-membered macrolides, streptogramins B and lincosamides remains unchanged. The easiest resistance to macrolides develops in such pathogens as pneumococcus, group A streptococci, *Haemophilus influenzae*. For example, in Taiwan in hospital strains of penicillin-resistant pneumococci the resistance to macrolides occurs in 90-95%. Community-acquired *Haemophilus influenzae* is resistant to clarithromycin; among the children in Germany there are 1-5% of cases, and group A streptococcus is found in about 4%. It is noted that the frequency of resistant strains increases every year. In this regard the use of macrolides should be rational [2, 7, 9, 12].

Clarithromycin is a macrolide that is the most resistant to hydrolysis in the acidic medium. This significantly increased its bioavailability, reduced the number of adverse events in the gastrointestinal tract (GIT) and made it practically independent of food intake. The drug is rapidly absorbed from the gastrointestinal tract, reaching the maximum concentrations in 1 (250 mg) or 2 hours (500 mg) – 1 and 2.41 mg/L, respectively; its bioavailability after oral administration is 55%. When taking different doses such indicators as C_{max} and AUC increase proportionally to the dose increase. The steady state concentrations of clarithromycin in the blood are created after re-taking of 5 doses. In the stationary phase the indicators of C_{max} of clarithromycin are equal 1-1.5 and 2-3 mg/L after administration of 250 or 500 mg, respectively. After re-taking of 200 mg twice daily for 14 days the accumulation of the drug in the blood is not observed. Clarithromycin has a low degree of ionization and is soluble in lipids, and therefore, it is well distributed in various organs and tissues. The volume of distribution of clarithromycin ranges from 115 to 266 litres, and binding with the serum proteins is from 42 to 70% [1, 3, 19].

Approximately 1/2 of the dose of clarithromycin is metabolized by microsomal liver enzymes to form the main metabolite – 14-hydroxy (R) of clarithromycin, which production is greater than that of 14-hydroxy (S) epimer. The antibacterial activity of 14-hydroxy clarithromycin (14-hydroxy CM) is little inferior to the predecessor, so the effect of the first passage through the liver almost does not impact on its activity, and parenteral administration has only some advantages. 14-Hydroxy CM *in vitro* equals erythromycin in its activity relative to *H.influenzae*. A hypothesis has been put forward that there is synergy concerning *Haemophilus influenzae* between clarithromycin and its metabolite, 14-hydroxy CM, and therefore, the activity of clarithromycin is higher *in vivo* than *in vitro*. However, this hypothesis is confirmed by the few studies that can not serve as a convincing base. The half-life of clarithromycin after a single dose is 2.6-4.6 hours; this indicator is higher for 14-hydroxy CM – 3.9-6.6 hours. The total clearance of clarithromycin ranges from 22 to 64 l/h. Urinary excretion of clarithromycin is 18-36%, and 14-hydroxy CM – 9.6-12%. The urine contains high concentrations of clarithromycin. Part of clarithromycin and its metabolite are excreted in faeces – 6.6 and 11.3%. Clarithromycin pharmacokinetics in children aged 6 months to 10 years is the same as in adults. Older people (65-84 years old) have higher C_{max} of clarithromycin, 14-hydroxy KM and AUC, but their renal clearance is lower than that of people aged 18-30. In patients with severe renal impairment increase in C_{max} of clarithromycin in the blood and AUC, $T_{½}$ prolongation and decrease of the elimination rate constant correlated with the degree of renal failure are observed. In patients with liver disease there are no significant changes in pharmacokinetics of clarithromycin, but there are some marked changes in such indicators as C_{max} and AUC of 14-hydroxy CM [1, 2, 4, 9].

The advantage of new macrolides is their ability to generate high and stable concentrations in tissues that exceed the level of the drug in the serum. It is known that clarithromycin achieves high concentrations in tissues and respiratory secretions. Peak concentrations of clarithromycin and its 14-hydroxy metabolite in the serum are lower than that of erythromycin (1.1 mg/L compared to 2.9±0.8 mg/l, respectively). Erythromycin and clarithromycin C_{max} is lower than MIC 90 for some important pathogens of respiratory tract infections, including erythromycin resistant pneumococci and *H.influenzae*. The concentration of clarithromycin created in various tissues, particularly in the tonsils, lungs, the prostate gland exceeds that of erythromycin, and far exceeds the concentration of the drug in the serum. The high concentration of clarithromycin in tissues, however, has a limited clinical value. This concentration was obtained in the homogenizate of solid tissues consisted mainly of the intracellular material, and their high level of concentration was due to high concentrations inside the cells. The high concentration of antibiotic inside the cells is important mainly for intracellular microorganisms, and less important in extracellular pathogens. Efficacy of macrolides relative to extracellular pathogens depends on the extracellular concentration of the antibiotic and the sensitivity of microorganisms to it. The time during which the concentration of free extracellular antibiotic exceeds the MIC value is the main factor that determines the effectiveness of macrolides [1, 2, 10, 17].

Permeability of clarithromycin increases when there is inflammation in the site. This is due to the fact that macrolides, in particular clarithromycin and azithromycin, have a special tropism to cells of the immune system. Thus, the ratio of the intracellular concentration of clarithromycin to the extracellular concentration for polymorphonuclear leukocytes is 20-38, for mononuclear cells – 16-24. As a result, macrophages loaded with clarithromycin during their migration transport the drug into the site of inflammation creating particularly high concentrations of the drug. It is believed that clarithromycin reaches relatively high (20-70 mg/L) concentrations in the fluid covering the epithelium (FCE), which is a complex biological fluids and inflammatory cells, and this fluid washes the terminal bronchioles and alveoles. The main difficulty in determining the antibiotic concentration in FCE is that when using the bronchoalveolar lavage, phagocytes that are present in the liquid

are placed in the antibiotic-free medium. In this case, as a result of osmosis, any antibiotic is quickly released from phagocytes. In one of the *in vitro* studies it has been shown that a significant number of many antibiotics that are in phagocytes can be excreted into the surrounding liquid for 20 minutes. Taking into account the artificial efflux of the intracellular antibiotic it is likely that the concentration of clarithromycin in the secretory fluid is somewhat exaggerated, but the true value is too low to be of therapeutic value. In general, clarithromycin in relation to distribution in the body occupies the "golden mean" – a balanced intermediate position among other macrolides [1, 10, 21].

The assumption of the presence of immunomodulatory properties of macrolide antibiotics was first expressed by the Japanese researchers in the 60's of the last century. The precondition for such assumptions was the results of using erythromycin in 1991 by Tanimoto H. in patients with diffuse panbronchiolitis, it significantly improved the survival rate of patients, including patients with the respiratory tract colonization by *P.aeruginosa* despite the fact that this organism was not included in the spectrum of activity of macrolides. Efficacy of treatment was associated with the nonantibacterial activity of macrolides as applicable dosage did not create bactericidal concentrations in the airways. In modern macrolides, in particular clarithromycin, the anti-inflammatory, immunomodulatory and mucoregulatory properties have been found. The primary mechanism for the immunomodulatory actions of macrolides today is considered to be their ability to affect the NF-kB- and MAPK-dependent (in particular, ERK1/2 cascade) signaling pathways of cells that are used to transfer information from the plasma membrane receptors to nuclear transcription factors. This mechanism can explain many immunomodulatory effects of macrolides, including inhibition of the mucus secretion, production of pro-inflammatory cytokines, chemotaxis and cell proliferation. Specific proteins or receptors that mediate the effects of macrolides on signaling pathways have not been identified yet [4, 17, 19, 22].

At present under immunomodulatory effects of macrolides we understand the whole spectrum of effects of these drugs on different levels of the body's immunological protection, including production of cytokines, the function of epithelial cells, etc. It should be emphasized that macrolides due to their properties are immunomodulators that activate specific immune defense mechanisms and simultaneously inhibit the excessive inflammation of the respiratory tract leading to fibrosis [1, 2, 10, 11].

Clarithromycin has the modulating effect on phagocytosis, chemotaxis, killing and apoptosis of neutrophils. The inhibition of the oxidative burst occurs, resulting in reduced formation of highly active compounds that can damage their own tissues. Decrease of synthesis and/or secretion of pro-inflammatory (IL-1, -6, -8, tumour necrosis factor (TNF)) and increase of secretion of pro-inflammatory cytokines (IL-2, -4, -10) were observed [8, 16, 21]. In 2013 A. Spyridaky with colleagues in a placebo-controlled double-blind study in patients with sepsis demonstrated reduction in TNF and IL-6 and growth of IL-10 in patients receiving clarithromycin compared to placebo. Thus, clarithromycin demonstrated ability to balance the level of pro- and anti-inflammatory interleukines [4].

In experimental models of ventilator-induced lung injury in rats it has been shown that inhibition of the nuclear factor kappa B (NFkB), which regulates gene expression of pro-inflammatory cytokines and TNF, is observed with the intravenous administration of clarithromycin. Compared to the control group the content of the serum TNF- and the oxidant status in animals treated with clarithromycin reduced significantly. The neutrophilic alveolar infiltration also decreased [5, 16, 20].

Clarithromycin has been found to reduce bronchial hyperreactivity and exhibit a beneficial effect on the clearance of bronchial and nasal secretions. This decreases the production of mucus in patients with excessive secretions, such as diffuse panbronchiolitis [13]. There is another significant aspect of the effectiveness of clarithromycin. It is in the impact on adhesion of bacteria to the epithelium, on products by microorganisms to pathogenicity factors, on formation of bacterial biofilms and quorum-sensing [10, 13, 14].

It is important that clarithromycin inhibits the formation of alginate biofilms created by *P.aeruginosa*. The probable mechanism of this action is inhibition of one of the enzymes involved in the synthesis of alginate – guanosine-D-mannose dehydrogenase. This anti-alginate and antibiofilm effect provides the clinical efficacy of clarithromycin in diffuse panbronchiolitis and cystic fibrosis since in the pathogenesis and clinic of these diseases the immune response caused by alginate of mucus is of substantial significance [7, 18]. For example, a 4-year intake of clarithromycin in the dose of 200 mg/day by patients with diffuse panbronchiolitis significantly improved clinical symptoms and functional performance by the 6th month of treatment, followed by positive dynamics during the entire period of therapy. Several studies demonstrated the effectiveness of clarithromycin in bronchial asthma marked by a significant decrease in the severity of clinical symptoms, the number of eosinophils in the blood and sputum, and reduced bronchial hyperresponsiveness compared to placebo [13]. The study of Nixon L. et al. demonstrated the ability of clarithromycin prescribed to 25 patients with chronic obstructive pulmonary disease (COPD) for 2 weeks in the dose of 500 mg twice a day to improve the respiratory function indicators and reduce the severity of clinical symptoms [15]. In the study of the dosage form with sustained release used within 7 days in 120 patients with COPD without exacerbation a significant decrease in the content of IL-8 in the sputum and reduction of its viscosity were observed [6, 22].

The research on *Pseudomonas aeruginosa* isolates in the form of biofilms obtained from patients with cystic fibrosis demonstrated a significant reduction in MIC of antipseudomonal antibacterial agents during the treatment with clarithromycin. Thus, the presence of additional features along with a high antibacterial activity provides a rapid regression of symptoms and improve-

ment of the patients' state when treating respiratory tract infections with clarithromycin [7, 10, 21].

A group of the Japanese researchers also studied the effect of clarithromycin on the migration of fibroblasts induced by the human plasma fibronectin, and the fetal lung fibroblast contraction of the human tissue. With a chemotactic tablet it was shown that clarithromycin inhibited the migration of fibroblasts ($p<0.05$), while other antibiotics (ampicillin, minocycline, and azithromycin macrolide) had no similar effect. The effect of clarithromycin on the migration of fibroblasts is of a dose-dependent nature. The drug also inhibits the migration of fibroblasts stimulated by the analogue of thromboxane A2. However, clarithromycin has no impact on another important function of fibroblasts – collagen gel contraction [11].

CONCLUSIONS

The results obtained have allowed to conclude that clarithromycin can be involved in the regulation of the wound healing process. The effectiveness of the indirect antimicrobial activity of clarithromycin is extensively studied *in vitro* and *in vivo*. This further expands our understanding of the therapeutic possibilities of this drug.

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АКТУАЛЬНІ ПИТАННЯ КЛІНІЧНОЇ ФАРМАКОЛОГІЇ КЛАРИТРОМІЦИНУ

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Ключові слова: *кларитроміцин; непрямі антибактеріальні ефекти; неантибактеріальні ефекти; імуномодулювальний вплив*

Останнім часом інтерес до кларитроміцину не зменшується, що підтверджується багаточисельними публікаціями та оглядами по цій темі. Даний препарат володіє широким профілем антибактеріальної активності, здатен проникати в клітини і створювати високі стабільні концентрації в тканинах, що перевищують рівень препарату в сироватці крові. Проте в останні роки більше уваги приділяється вивченню неантибактеріальних ефектів кларитроміцину. У сучасних макролідів, зокрема у кларитроміцину, виявлені протизапальні, імуномодулюючі та мукорегулюючі властивості. Первинним механізмом імуномодулюючої дії макролідів на сьогоднішній день прийнято вважати їх здатність впливати на NF-kB-та МАРК-залежні сигнальні шляхи клітин. Під впливом кларитроміцину відзначено зниження синтезу та / або

секреції прозапальних (IL-1, -6, -8, фактора некрозу пухлин (TNF)) і посилення секреції протизапальних цитокінів (IL-2, -4, -10). Ефективність непрямої антимікробної дії кларитроміцину інтенсивно вивчається in vitro та in vivo, що ще більше розширює наші уявлення про терапевтичні можливості даного препарату.

АКТУАЛЬНЫЕ ВОПРОСЫ КЛИНИЧЕСКОЙ ФАРМАКОЛОГИИ КЛАРИТРОМИЦИНА

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Ключевые слова: *кларитромицин; непрямые антибактериальные эффекты; неантибактериальные эффекты; иммуномодулирующее влияние*

В последнее время интерес к кларитромицину не уменьшается, что подтверждается многочисленными публикациями и обзорами по этой теме. Данный препарат обладает широким профилем антибактериальной активности, способен проникать в клетки и создавать высокие стабильные концентрации в тканях, превышающие уровень препарата в сыворотке крови. Однако в последние годы больше внимания уделяется изучению неантибактериальных эффектов кларитромицина. У современных макролидов, в частности у кларитромицина, обнаружены противовоспалительные, иммуномодулирующие и мукорегулирующие свойства. Первичным механизмом иммуномодулирующего действия макролидов на сегодняшний день принято считать их способность влиять на NF-kB-и МАРК-зависимые сигнальные пути клеток. Под влиянием кларитромицина отмечено снижение синтеза и / или секреции провоспалительных (IL-1, -6, -8, фактора некроза опухолей (TNF)) и усиление секреции противовоспалительных цитокинов (IL-2, -4, -10). Эффективность непрямого антимикробного действия кларитромицина интенсивно изучается in vitro и in vivo, что еще больше расширяет наши представления о терапевтических возможностях данного препарата.

Recommended by Doctor of Medicine, professor A.I.Bereznyakova

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THE STUDY OF THE ANTIHYPOXIC ACTION OF 1-PHENETHYL-5,7-DIHYDRO-1H-PYRROLO-[2,3-d]PYRIMIDIN-2,4,6-TRIONE (DEZAPUR) ON DIFFERENT MODELS OF HYPOXIA

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Key words: hypoxia; 1-phenethyl-5,7-dihydro-1H-pyrrolo-[2,3-d]pyrimidin-2,4,6-trione; antihypoxant drug Mexidol

Extension of hypoxic states is a consequence of disorder of cerebral, coronary and peripheral circulation, and it requires further search for substances that can reduce the negative effect of hypoxia on tissues, and increase their resistance to hypoxia. The study of the antihypoxic activity of 1-phenethyl-5,7-dihydro-1H-pyrrolo-[2,3-d]pyrimidin-2,4,6-trione under the conditional name of Dezapur on different models of hypoxia (hemic, hypercapnic, histotoxic and hypobaric) has been presented. The reference drug is Mexidol. Dezapur in the dose of 10 mg/kg and Mexidol in the dose of 100 mg/kg show the expressed antihypoxic activity on all models of hypoxia compared to the control group: hemic hypoxia in 2.09 and 1.82 times, hypercapnic hypoxia – in 2.35 and 2.29 times, on histotoxic hypoxia – in 2.04 and 1.9 times, hypobaric hypoxia – in 3.06 and 2.72 times, respectively.

Hypoxia is a pathological process, which is characterized by decrease of the oxygen content in the blood and tissues, development of the complex of the secondary non-specific metabolic and functional disorders, as well as the reaction of adaptation [1]. Extension of hypoxic states is a consequence of disorder of cerebral, coronary and peripheral circulation, and it requires further search for substances that can reduce the negative effect of hypoxia on tissues, and increase their resistance to hypoxia. Therefore, the search of such substances that would increase the resistance of tissues to hypoxia, bind free radicals formed as a result of hypoxia, i.e. having the antioxidant effect and reducing inflammatory edema, is a topical issue of medical chemistry [2, 5].

The study of active antihypoxant drugs is a current problem in pharmacology. Thus, the screening studies of 1-phenethyl-5,7-dihydro-1H-pyrrolo-[2,3-d]pyrimidin-2,4,6-trione (Dezapur), which can be a potential antioxidant drug, have been conducted [3].

Materials and Methods

The antihypoxic activity was studied on nonlinear white male mice weighing 20±2g. The test substance in the dose of 1/10 of its LD_{50} and the reference drug Mexidol in the dose of 100 mg/kg (ED_{50}) were introduced intragastrically 30 min prior to the experiment. Control and experiments were performed simultaneously and recorded the life time in minutes [7]. All animals were divided into 3 groups, each group of 6 animals: group 1 – intact animals received distilled water in the volume of 1 ml; group 2 – animals received Dezapur in the effective dose of 10 mg/kg; group 3 – animals received the reference drug Mexidol [6, 9].

The acute hypobaric hypoxia was created by raising animals to the height of 11.000 m and with the speed of 50 m/s in the Komovsky apparatus; the acute hypoxic hypoxia – by placing animals in a 200 ml airtight chamber; the hemic acute hypoxia – by subcutaneous injection of sodium nitrite in the dose of 225 mg/kg; the histotoxic hypoxia – by intraperitoneal introduction of sodium nitroprusside in the dose of 25 mg/kg. The antihypoxic action was assessed by duration of the animals' life. The results of the study were compared with the reference drug Mexidol widely known in medical practice as an antihypoxant and antioxidant drug [4, 8, 10].

Results and Discussion

According to the results obtained Dezapur exhibited a stable antihypoxic activity by the life expectancy of the animals on all experimental models (Table, Fig.). Its prophylactic introduction prolonged the life of mice in 2.0-3.1 times compared to the control group.

On the experimental models of the acute normobaric hypoxia and the acute histotoxic hypoxia the antihypoxic activity of Dezapur and Mexidol was almost identical. When introducing Dezapur on these models of hypoxia the life of animals was 2.5 and 2.1 times longer, and it was not significantly different from the indicators of Mexidol (2.4 and 2.0 times, respectively). The results obtained are statistically unreliable ($p>0.05$).

In the acute hemic hypoxia the efficiency of Dezapur exceeded the similar effect of the reference drug in 1.19 times. The life of animals was 1.97 times longer when introducing Dezapur and 1.65 times longer when introducing Mexidol.

Dezapur and Mexidol showed the highest antihypoxic activity in relation to the control group on the model of the acute hypobaric hypoxia. At the same time the efficiency of Dezapur was 1.13 times higher than that of Mexidol by the life expectancy in mice.

The data obtained concerning Mexidol coincide with the results of other authors [6, 9].

Table

The effect of Dezapur on the survival of white mice under conditions of different models of hypoxia (M±m), n=6

Conditions of the experiment	Acute hemic hypoxia, min	Normobaric hypercapnic hypoxia, min	Acute histotoxic hypoxia, min	Acute hypobaric hypoxia, min
Dezapur	27.38±0.72*	31.22 ±0.21*	12.82±0.52*	21.97±0.58*
Mexidol	23.82±0.61*	30.40±0.26*	11.95±0.42*	19.51±0.72*
Control	13.11±0.73	13.33±0.61	6.28 ±0.96	7.18±0.25

Notes: * – p<0.05 compared to the control group. The survival of control animals is taken as 100%.

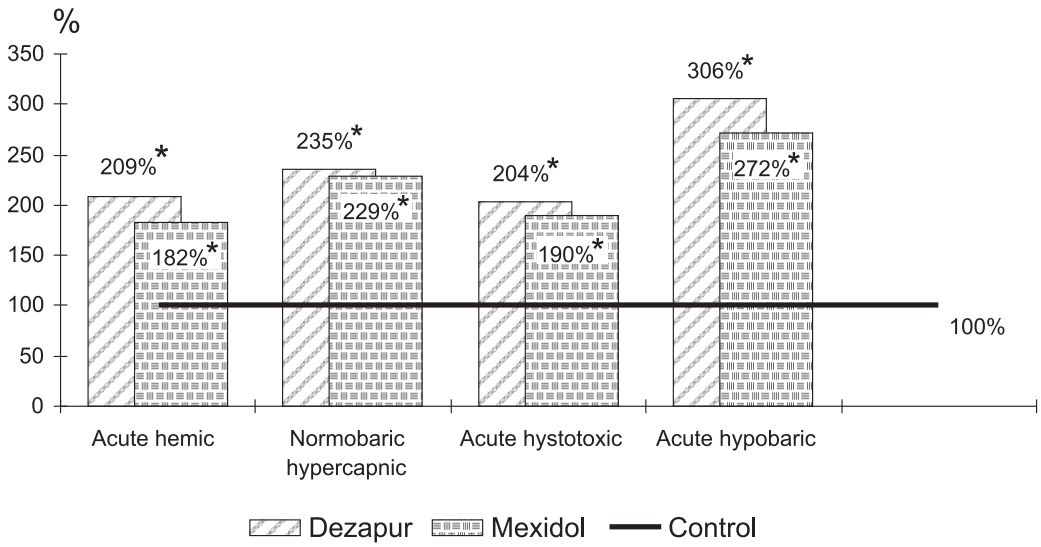

Fig. Duration of the animals' life under conditions of different types of the acute hypoxia in prophylactic introduction of Dezapur, %: * – p<0.05 compared to the control group.

The data obtained indicate that Dezapur in the dose of 10 mg/kg and Mexidol in the dose of 100 mg/kg show the expressed antihypoxic activity on all models of hypoxia compared to the control group: hemic hypoxia in 2.09 and 1.82 times, hypercapnic hypoxia – in 2.35 and 2.29 times, on histotoxic hypoxia – in 2.04 and 1.9 times, hypobaric hypoxia – in 3.06 and 2.72 times, respectively.

CONCLUSIONS

Under conditions of hemic, normobaric, histotoxic and hypobaric hypoxia (in white mice) Dezapur (10 mg/kg, single intragastric introduction) significantly prolongs the life of animals in 2.1; 2.0; 2.4; 3.1 times, respectively, and in this respect it is not inferior to the reference drug Mexidol. It indicates the presence of significant antihypoxant properties in the compound.

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ВИВЧЕННЯ АНТИГІПОКСИЧНОЇ АКТИВНОСТІ 1-ФЕНЕТИЛ-5,7-ДИГІДРО-1Н-ПІРОЛО[2,3-d]ПІРИМІДИН-2,4,6-ТРИОНУ НА РІЗНИХ МОДЕЛЯХ ГІПОКСІЙ

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Ключові слова: *гіпоксія; 1-фенетил-5,7-дигідро-1Н-піроло[2,3-d]піримідин-2,4,6-трион; антигіпоксант мексидол*

Розповсюдження гіпоксичних станів є наслідком порушення мозкового, коронарного та периферичного кровообігу, що потребує подальшого пошуку речовин, які здатні зменшувати негативний вплив гіпоксії на тканини, підвищуючи їх стійкість до гіпоксії. Представлені дослідження антигіпоксичної активності 1-фенетил-5,7-дигідро-1Н-піроло[2,3-d]піримідин-2,4,6-триону (умовна назва «Дезапур») на різних моделях гіпоксій (гемічній, гіперкапнічній, гістотоксичній та гіпобаричній) у співставленні з препаратом порівняння – антигіпоксантом мексидолом. Дезапур у дозі 10 мг/кг та мексидол у дозі 100 мг/кг проявили виражену антигіпоксичну активність на всіх видах гіпоксій по відношенню до контролю – на гемічній гіпоксії в 2,09 та 1,82, на гіперкапнічній – в 2,35 та 2,29, на гістотоксичній – в 2,04 та 1,9, на гіпобаричній – в 3,06 та 2,72 рази відповідно.

ИЗУЧЕНИЕ АНТИГИПОКСИЧЕСКОЙ АКТИВНОСТИ 1-ФЕНЕТИЛ-5,7-ДИГИДРО-1Н-ПИРРОЛО[2,3-d]ПИРИМИДИН-2,4,6-ТРИОНА НА РАЗНЫХ МОДЕЛЯХ ГИПОКСИЙ

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Ключевые слова: *гипоксия; 1-фенетил-5,7-дигидро-1Н-пирроло[2,3-d]пиримидин-2,4,6-трион; антигипоксант мексидол*

Распространение гипоксических состояний является следствием нарушения мозгового, коронарного и периферического кровообращения, что требует дальнейшего поиска веществ, способных уменьшать негативное влияние гипоксии на ткани, повышая их устойчивость к гипоксии. Представлены исследования антигипоксической активности 1-фенетил-5,7-дигидро-1Н-пирроло[2,3-d]пиримидин-2,4,6-триона (условное название «Дезапур») на разных моделях гипоксий (гемической, гиперкапнической, гистотоксической, гипобарической). Препарат сравнения – мексидол. Дезапур в дозе 10 мг/кг и мексидол в дозе 100 мг/кг проявили выраженную антигипоксическую активность на всех видах гипоксий по отношению к контролю – на гемической гипоксии в 2,09 и 1,82, на гиперкапнической – в 2,35 и 2,29, на гистотоксической – в 2,04 и 1,9, на гипобарической – в 3,06 и 2,72 раза соответственно.

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