



**ДО 75-РІЧЧЯ РЕКТОРА НАЦІОНАЛЬНОГО
ФАРМАЦЕВТИЧНОГО УНІВЕРСИТЕТУ,
ДІЙСНОГО ЧЛЕНА (АКАДЕМІКА)
НАЦІОНАЛЬНОЇ АКАДЕМІЇ НАУК УКРАЇНИ,
ЛАУРЕАТА ДЕРЖАВНОЇ ПРЕМІЇ УКРАЇНИ,
ДОКТОРА ФАРМАЦЕВТИЧНИХ НАУК,
ДОКТОРА ХІМІЧНИХ НАУК, ПРОФЕСОРА
ВАЛЕНТИНА ПЕТРОВИЧА ЧЕРНИХ**

5 січня 2015 року виповнилося 75 років ректору Національного фармацевтичного університету Валентину Петровичу Черних, дійсному члену (академіку) Національної академії наук України, лауреату Державної премії України, доктору фармацевтичних наук, доктору хімічних наук, професору. 2015 рік знаменний для Валентина Петровича ще й тим, що виповнюється 50 років його науково-педагогічної та громадської діяльності і 35 років перебування на посаді ректора.

Півстоліття життя віддано служінню благородній місії – підготовці фахівців для фармацевтичної галузі, підготовці наукових і науково-педагогічних кадрів, перебудові та реорганізації Національного фармацевтичного університету – головного фармацевтичного вищого навчального закладу України з історією, яка починалася у далекому 1805 році, реформуванню вищої фармацевтичної освіти та фармацевтичної галузі України.

В.П.Черних пройшов шлях від студента, аспіранта, асистента, доцента, професора, завідувача кафедри, декана, проректора з навчальної роботи до ректора Національного фармацевтичного університету.

Під керівництвом В.П.Черних Харківський фармацевтичний інститут пройшов складні етапи реорганізації від невеликого, маловідомого інституту до Національного фармацевтичного університету, який відповідає найвищим державним і міжнародним критеріям.

Сьогодні колектив університету налічує понад 20 тисяч співробітників і студентів. Під керівництвом видатного організатора Харківський фармацевтичний інститут, в якому навчалися 1600 студентів за однією спеціальністю «Фармація» та працювали 6 докторів наук і 73 кандидати наук, виріс в унікальний науково-освітній комплекс – Національний фармацевтичний університет, в якому сьогодні навчаються 17 500 студентів за 14 спеціальностями та здійснюють науково-педагогічну діяльність 110 докторів наук і 500 кандидатів наук, середній вік яких становить 45 років. У 1991 р. Харківський фармацевтичний інститут одним із перших серед 900 ВНЗ отримав статус акредитованого на союзному рівні. У 1999 р. у першій п'ятірці ВНЗ України він набув статусу національного і став другим національним ВНЗ у м. Харкові.

Під керівництвом В.П.Черних здійснений кадровий «прорив» у НФаУ: з 1980 року підготовлено більше 130 докторів наук і 650 кандидатів наук. За рейтингом ЮНЕСКО серед 200 кращих університетів України НФаУ має один із найвищих показників якості науково-педагогічного потенціалу – 94%. За останні 15 років у НФаУ відкрито 13 нових спеціальностей, Інститут підвищення кваліфікації спеціалістів фармації, коледж. Протягом усього періоду управління університетом В.П.Черних сприяв забезпеченню його стабільного фінансового становища, створенню ефективної системи соціального захисту співробітників і студентів. НФаУ посідає лідерські позиції в Україні, в національному рейтингу він знаходиться на 2-му місці серед 18 медичних навчальних закладів і на третьому серед харківських університетів. Університет є флагманом фармацевтичної освіти серед навчальних закладів країн СНД. НФаУ нагороджений Почесною грамотою Кабінету Міністрів України за вагомий внесок у розвиток медичної та фармацевтичної науки і освіти. Це

університет європейського рівня, визнаний у світі, який є дійсним членом міжнародних фармацевтичних та освітніх асоціацій. У 2013 р. НФаУ приєднався до Великої Хартії університетів. Будучи спеціалізованим ВНЗ, він забезпечує комплексну підготовку фахівців високої якості за всіма напрямками фармацевтичної галузі. У його аудиторіях отримали вищу фармацевтичну освіту понад 50 тисяч фахівців, серед яких понад 6 тисяч магістрів фармації для 82 країн світу. Підготовка фахівців для зарубіжжя – це вагомий чинник підвищення міжнародного іміджу нашої держави та освіти.

З метою реалізації державної політики кадрового забезпечення галузі В.П.Черних запропонував систему підготовки фахівців «на місцях» шляхом відкриття мережі з 20 фармацевтичних факультетів при медичних ВНЗ і забезпечення їх науково-педагогічними кадрами та навчально-методичною літературою. В університеті здійснюється підготовка науково-педагогічних кадрів для фармацевтичних факультетів ВНЗ, практичної фармації України та зарубіжних країн.

Уперше в системі фармацевтичної освіти України створені навчально-методичні комплекси навчальної літератури з усіх дисциплін обсягом понад 2 тис. найменувань. Навчальний процес на 100% забезпечений навчально-методичною літературою державною та іноземними мовами, яка використовується на всіх фармацевтичних факультетах України та деяких країн СНД. Наукова спадщина університету – це понад 490 підручників і навчальних посібників, 300 монографій, більше 1500 охоронних документів на винаходи. Вченими НФаУ розроблено і впроваджено у виробництво 261 новий лікарський препарат.

В.П.Черних є ініціатором і одним з авторів розробки Концепції розвитку фармацевтичної галузі та освіти України, розширення спектра спеціальностей для фармацевтичної галузі, основоположником новітнього напрямку в фармації: фармацевтичної опіки хворих, системи контролю якості ліків, у т.ч. впровадження біоеквівалентності на засадах належної клінічної практики відповідно до світових вимог.

Для підвищення авторитету та визнання на державному рівні фармацевтичної галузі за ініціативи та безпосередньої участі В.П.Черних в Україні встановлено професійне свято – День фармацевтичного працівника (з 1999 р.), запроваджено нову державну нагороду – почесне звання «Заслужений працівник фармації України» (2005 р.), прийнято Етичний кодекс фармацевтичного працівника України (2010 р.), створено першу в світі Фармацевтичну енциклопедію (перше видання – 2005 р., друге – 2010 р.). Під безпосереднім керівництвом В.П.Черних культурна скарбниця Харківщини збагатилася унікальною скульптурною композицією «Фармація у віках» – першим у світі пам'ятником фармацевту. В.П.Черних став ідеологом зміцнення галузі та організатором проведення на базі університету V, VI і VII Національних з'їздів фармацевтів України і створення Фармацевтичної асоціації України.

В.П. Черних – видатний вчений в галузі органічної хімії, праці якого широко відомі науковій спільноті України і зарубіжжя, є автором 1260 наукових праць, серед яких перший підручник для вищої фармацевтичної освіти України «Органічна хімія» в 3-х томах, удостоєний Державної премії України в галузі науки і техніки в 2000 р. За його ініціативи заснований новий науковий напрям – синтез біологічно активних речовин – похідних дикарбонових кислот, створення на їх основі різних гетероциклічних структур і дослідження шляхів циклізації поліфункціональних реагентів в ансамблі гетероциклів. Новизну і пріоритетність наукових досліджень підтверджують 126 патентів України та Росії, 341 авторське свідоцтво. Більше 40 років віддано підготовці докторів і кандидатів наук для вищої школи і практичної фармації, створена вітчизняна школа хіміків-синтетиків, у рамках якої вченим підготовлено понад 60 докторів і кандидатів наук, а також (особисто та з учнями) створено 16 лікарських препаратів.

За підсумками багаторічних наукових досліджень в області синтезу біологічно активних речовин у 1997 р. професор В.П.Черних був обраний членом-кореспондентом НАН України. В історії фармації України ця подія стала першим прикладом представництва фармації в академічній науці. За наукові досягнення Президія академії наук України нагородила В.П.Черних в 2013 р. почесним знаком НАНУ.

В.П. Черних – відомий державний і громадський діяч, ініціатор видання 7 наукових журналів ВАК України. Впродовж 30 років він працював в Експертних радах ВАК СРСР та України. На теперішній час він очолює Експертну проблемну комісію «Фармація» МОЗ та НАМН України, є го-

ловою Науково-методичної комісії з фармації Міністерства освіти і науки України, членом Вченої ради ДП «Державний фармакологічний центр» МОЗ України, членом Президії Фармакопейного Комітету МОЗ України, членом Вченої медичної ради МОЗ України, членом бюро Державного фармакологічного центру з реєстрації ЛЗ і ЛП, членом секції хімії та хімічної технології Комітету з Державних премій в галузі науки і техніки, членом колегії Держінспекції з контролю якості лікарських препаратів МОЗ України. В.П.Черних – віце-президент Фармацевтичної асоціації України, президент Фармацевтичної асоціації Харківщини. Обирався депутатом Київської районної ради народних депутатів м. Харкова (1986 р.) і міської Ради народних депутатів (1985-1987 рр.). У 1999 р. Міжнародний біографічний центр та Американський біографічний інститут визнали В.П.Черних одним із 500 найбільш впливових і видатних учених світу, який здійснює активну міжнародну та просвітницьку діяльність.

Плідна праця та видатні заслуги відомого вченого, педагога, організатора, державного і громадського діяча були неодноразово відзначені державою: він нагороджений орденами «Знак пошани», «Трудового Червоного Прапора», орденами України «За заслуги» I, II, III ступенів, князя Ярослава Мудрого IV і V ступенів, Почесною грамотою Верховної Ради України, почесними грамотами та відзнаками МОЗ та МОН України «Відмінник охорони здоров'я», «Відмінник освіти України», «Винахідник СРСР», «Петро Могила», відзнакою Харківської облдержадміністрації «Слобожанська слава», йому були присвоєні почесні звання «Заслужений винахідник УРСР», «Заслужений діяч науки і техніки УРСР». Харківська громадськість обрала В.П.Черних Почесним громадянином м. Харкова.

Науково-педагогічна та академічна громадськість, колектив і студенти Національного фармацевтичного університету, колеги, друзі, учні від щирого серця вітають відомого вченого, талановитого педагога, видатного організатора і реформатора вищої фармацевтичної освіти, невтомного ентузіаста і патріота фармації, який є яскравим прикладом відданого служіння інтересам освіти, науки, здоров'я людей, інтересам нашої славної України.

Нових Вам, Валентине Петровичу, звершень і злетів, невичерпного творчого натхнення та довголіття!

Коли верстався номер!

6 березня 2015 року на загальних зборах НАН України ректора Національного фармацевтичного університету професора Черних В.П. було обрано дійсним членом (академіком) НАН України зі спеціальності хімія лікарських сполук. Це знакова подія в житті університету і фармації в цілому.

Шановний Валентине Петровичу! Фармацевтична спільнота, викладачі та студенти Національного фармацевтичного університету щиро вітають Вас з цією знаменною подією і бажають доброго здоров'я, нових досягнень та відкриттів!

СИНТЕЗ ТА АНАЛІЗ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН

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

UDC 547.732:547.853:547.39'054.4:66.095.253

SYNTHESIS AND THE STUDY OF THE ANTIMICROBIAL ACTIVITY OF 3-AMINO-5-METHYL-2-(ALKYLTHIO)-4-OXO-N-ARYL-3,4-DIHYDROTHIENO[2,3-*d*]PYRIMIDINE-6-CARBOXAMIDES

S.V.Vlasov, S.M.Kovalenko, T.P.Osolodchenko, V.P.Chernykh

National University of Pharmacy

Institute of Microbiology and Immunology named after I.I.Mechnikov at NAMS of Ukraine

Key words: thiophene; pyrimidine; amides; alkylation

*By alkylation of 3-amino-5-methyl-4-oxo-N-aryl-2-thioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-6-carboxamides with substituted benzylchlorides and chloroacetic acid the series of novel derivatives of 3-amino-5-methyl-2-(alkylthio)-4-oxo-N-aryl-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxamides have been obtained. The structures of these compounds have been confirmed by ¹H NMR spectral data and the elemental analysis. For all of the products obtained the ¹H NMR spectra contain the signals of the amino group as the sharp singlet in the region of 5.75-5.84 ppm, the signal of the methyl group at position 5 of the thieno[2,3-*d*]pyrimidine system (2.68-2.75 ppm); the signal of the carboxamide NH group, which position varies from 9.67 ppm to 10.61 ppm depending on the structure of the substituent of the amide aromatic cycle, is also observed. In the spectra the signals of the benzyl CH₂ group protons are located in the region of 4.23-4.29 ppm, while the same signal for the derivative of thioacetic acid is observed at 3.82 ppm. The antimicrobial activity screening for the compounds obtained has been performed by the agar well diffusion method. In general, it has been found that all of the compounds tested appeared to be more active than the reference drugs against the strains of both *Proteus vulgaris* and *Pseudomonas aeruginosa*; 3-amino-2-(benzylthio)-5-methyl-N-(4-methylphenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxamide was the most active compound, which moderately inhibited the growth of all test-strains of microorganisms, and showed even higher activity than the reference drugs streptomycin and metronidazole against *Bacillus subtilis* and *Candida albicans* fungi.*

It was reported that some derivatives of thieno[2,3-*d*]pyrimidin-4(1*H*)-one showed the antimicrobial activity [1-4, 6, 8-10]. The compounds with modified amino and thioxo groups at positions 2 and 3 of the core heterocyclic system [3], as well as the derivatives containing these groups included into the other fused heterocyclic system [1, 6, 9] are known among them. Our special attention was paid to the derivatives containing the carboxamide group at α -position of the thiophene ring. Earlier we reported about the triheterocyclic systems containing similar heterocyclic fragments as the effective antimicrobials [1, 9]. However, the simpler compounds with the free amino group at position 3 were not prepared previously and were not used for antimicrobial trials. Therefore, the synthesis and study of the antimicrobial activity of derivatives of 3-amino-5-methyl-2-(alkylthio)-4-oxo-N-aryl-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxamide were the aim of our research work.

Materials and Methods

Chemical Part

All of the solvents and reagents were received from the commercial sources. Melting points (°C) were de-

termined with a Kofler (Hotbench) melting point apparatus. ¹H NMR spectra were recorded using a Varian Mercury (200 MHz) spectrometer in DMSO-*d*₆ with TMS as an internal standard. Chemical shifts (δ) are reported in ppm. Elemental analysis was performed by Kjeldahl method.

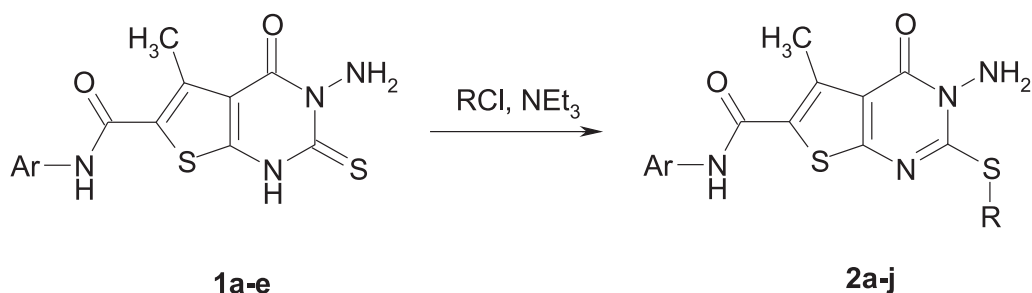
3-Amino-5-methyl-4-oxo-N-phenyl-2-thioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-6-carboxamides (1) were obtained by the methods previously reported [1, 9].

The general method for the synthesis of 3-amino-5-methyl-2-(alkylthio)-4-oxo-N-aryl-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxamides (2)

To the suspension of 0.00045 mol of the starting compound **1** in dimethylformamide add 0.0005 mol of triethylamine and 0.0005 mol of the corresponding chloroderivative. Heat the reaction mixture (70°C) with stirring for 5-8 hours. After cooling the reaction mixture dilute it with water. Filter the precipitate of compound **2** formed and wash with water. Purify the products by boiling in 2-propanol.

The study of the antimicrobial activity

The microbiological experiment was performed at the premises of the laboratory of Biochemistry of Mic-



1a: Ar = 2-Pyridyl; **1b:** Ar = 2-MeC₆H₄; **1c:** Ar = 4-MeC₆H₄; **1d:** Ar = 4-FC₆H₄; **1e:** 4-CH₃OC₆H₄; **2a:** Ar = 2-Pyridyl, R = C₆H₅CH₂;
2b: Ar = 2-Pyridyl, R = 4-CH₃C₆H₄CH₂; **2c:** Ar = 2-MeC₆H₄, R = C₆H₅CH₂; **2d:** Ar = 4-MeC₆H₄, R = C₆H₅CH₂;
2e: Ar = 4-MeC₆H₄, R = 4-CH₃C₆H₄CH₂; **2f:** Ar = 4-MeC₆H₄, R = HOOCCH₂; **2g:** Ar = 4-FC₆H₄, R = C₆H₅CH₂;
2h: Ar = 4-FC₆H₄, R = 4-CH₃C₆H₄CH₂; **2i:** Ar = 4-CH₃OC₆H₄, R = C₆H₅CH₂; **2j:** Ar = 4-CH₃OC₆H₄, R = 4-CH₃C₆H₄CH₂.

Scheme

roorganisms and Nutrient Media at the Institute of Microbiology and Immunology named after I.I.Mechnikov at NAMS of Ukraine. According to the WHO recommendations [5, 7] such test-strains as *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 4636, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC653/885 were used. Bacterial concentration was 10⁷ CFU/ml (determined by McFarland standard). Overnight cultures kept for 18-24 h at 36°C±1°C were used. The bacterial suspension was inoculated onto the entire surface of a Mueller-Hinton agar (Dagestan Research Institute of Nutrient Media). The compounds were introduced to the wells in the form of DMSO solution in concentrations of 100 µg/ml; the open wells were filled with 0.3 ml of the solution.

Results and Discussion

The target compounds **2** (scheme) were obtained by alkylation of 3-amino-5-methyl-4-oxo-*N*-aryl-2-thioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-6-carboxamide derivatives **1** with the substituted benzyl chlorides and chloroacetic acid. Starting compounds **1 a-e** were obtained by the methods previously reported [1, 9], and their purity appeared to be sufficient for further transformations. All of the compounds **2** were isolated as crystalline solids (Table 1).

The structure of the compounds obtained was confirmed by ¹H NMR spectroscopic method (Table 2). For all of the compounds **2** their ¹H NMR spectra contain the signals of the amino group as the sharp singlet in the region of 5.75-5.84 ppm, the signal of the methyl group at position 5 of the thieno[2,3-*d*]pyrimidine sys-

Table 1

Physico-chemical properties of 3-amino-5-methyl-2-(alkylthio)-4-oxo-*N*-aryl-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxamides (**2a-j**)

Compd. No.	Ar	R	Mol. formula M.w.	Yield %, in the alkylation step	M.p., °C	N%
						calc. found
2a	2-Pyridyl	C ₆ H ₅ CH ₂	C ₂₀ H ₁₇ N ₅ O ₂ S ₂ 423.52	79	264-266	16.54 16.67
2b	2-Pyridyl	4-CH ₃ C ₆ H ₄ CH ₂	C ₂₁ H ₁₉ N ₅ O ₂ S ₂ 437.55	83	216-217	16.01 16.23
2c	2-MeC ₆ H ₄	C ₆ H ₅ CH ₂	C ₂₂ H ₂₀ N ₄ O ₂ S ₂ 436.56	77	25-256	12.83 12.90
2d	4-MeC ₆ H ₄	C ₆ H ₅ CH ₂	C ₂₂ H ₂₀ N ₄ O ₂ S ₂ 436.56	69	240-241	12.83 13.02
2e	4-MeC ₆ H ₄	4-CH ₃ C ₆ H ₄ CH ₂	C ₂₃ H ₂₂ N ₄ O ₂ S ₂ 450.59	83	256-258	12.43 12.38
2f	4-MeC ₆ H ₄	CH ₂ COOH	C ₁₇ H ₁₆ N ₄ O ₄ S ₂ 404.47	53	189-190	13.85 13.97
2g	4-FC ₆ H ₄	C ₆ H ₅ CH ₂	C ₂₁ H ₁₇ FN ₄ O ₂ S ₂ 440.52	69	278-279	12.72 12.75
2h	4-FC ₆ H ₄	4-CH ₃ C ₆ H ₄ CH ₂	C ₂₂ H ₁₉ FN ₄ O ₂ S ₂ 454.55	75	297-299	12.33 12.56
2i	4-CH ₃ OC ₆ H ₄	C ₆ H ₅ CH ₂	C ₂₂ H ₂₀ N ₄ O ₃ S ₂ 452.56	83	252-253	12.38 12.49
2j	4-CH ₃ OC ₆ H ₄	4-CH ₃ C ₆ H ₄ CH ₂	C ₂₃ H ₂₂ N ₄ O ₃ S ₂ 466.58	88	289-291	12.01 12.12

Table 2

Data of ^1H NMR-spectra of 3-amino-5-methyl-2-(alkylthio)-4-oxo-*N*-aryl-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxamides (**2a-j**)

Compd. No.	Chemical shift, δ , ppm.				
	NH_2 (2H, s)	Thiophene CH_3 (3H, s)	NH (1H, br.s)	Aliphatic protons	Aromatic protons
2a	5.76	2.72	10.59	4.29 (2H, s, CH_2);	7.16 (1H, t); 7.29 (3H, m); 7.43 (2H, m); 7.82 (1H, t); 8.05 (1H, d); 8.36 (1H, d)
2b	5.76	2.72	10.61	2.25 (3H, s, CH_3); 4.24 (2H, s, CH_2);	7.13 (3H, m); 7.31 (2H, d); 7.82 (1H, t); 8.05 (1H, d); 8.36 (1H, d)
2c	5.76	2.68	10.08	2.26 (3H, s, CH_3); 4.28 (2H, s, CH_2);	7.14 (2H, d); 7.29 (3H, m); 7.44 (2H, m); 7.53 (2H, d)
2d	5.76	2.75	9.67	2.23 (3H, s, CH_3); 4.29 (2H, s, CH_2);	7.09-7.48 (9H, m)
2e	5.76	2.68	10.10	2.25 (6H, s, 2CH_3); 4.23 (2H, s, CH_2);	7.13 (4H, m); 7.31 (2H, d); 7.54 (2H, d)
2f	5.84	2.68	10.07	2.25 (3H, s, CH_3); 3.82 (2H, s, CH_2);	7.12 (2H, d); 7.52 (2H, d)
2g	5.76	2.70	10.22	4.29 (2H, s, CH_2);	7.09-7.50 (6H, m); 7.68 (2H, m)
2h	5.75	2.70	10.21	2.25 (3H, s, CH_3); 4.24 (2H, s, CH_2);	7.15 (4H, m); 7.31 (2H, m); 7.67 (2H, m)
2i	5.76	2.69	10.03	3.72 (3H, s, OCH_3); 4.28 (2H, s, CH_2);	6.90 (2H, d); 7.29 (3H, m); 7.44 (2H, m); 7.56 (2H, d)
2j	5.76	2.69	10.04	2.25 (3H, s, CH_3); 3.72 (3H, s, OCH_3); 4.23 (2H, s, CH_2);	6.90 (2H, d); 7.10 (2H, d); 6.32 (2H, d); 7.56 (2H, d);

tem (2.68-2.75 ppm); the signal of the carboxamide NH group, which position varies from 9.67 ppm to 10.61 ppm depending on the structure of the substituent of the amide aromatic cycle, is also observed. In the spectra the signals of benzyl CH_2 group protons are located in the region of 4.23-4.29 ppm, while the same signal for the derivative of thioacetic acid is observed at 3.82 ppm.

As to the spectrum and efficacy of antimicrobial properties, the highest activity in the range of compounds **2** (Table 3) was revealed by compound **2d**, which

moderately inhibited the growth of all test-strains of microorganisms and showed even higher activity than the reference drugs against *Bacillus subtilis* and *Candida albicans* fungi. All of the 4-methylphenyl amides **2e** and **2f** tested also exhibited a moderate antimicrobial activity. Its noteworthy that most of the compounds studied, namely **2a**, **2d-2g** and **2i** appeared to be more active than the reference drugs against *Proteus vulgaris* and *Pseudomonas aeruginosa*; it agreed with the previously reported typical manner of thieno[2,3-*d*]pyrimidines [10].

Table 3

The antimicrobial activity of 3-amino-5-methyl-2-(alkylthio)-4-oxo-*N*-aryl-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxamides (**2a-j**)*

Compnd. No.	<i>Staphylococcus aureus</i> ATCC 25923	<i>Escherichia coli</i> ATCC 25922	<i>Proteus vulgaris</i> ATCC 4636	<i>Pseudomonas aeruginosa</i> ATCC 27853	<i>Bacillus subtilis</i> ATCC 6633	<i>Candida albicans</i> ATCC 653/885
2a	++	++	+	+	++	+
2b	++	+	–	+	++	+
2c	–	–	–	–	–	–
2d	++	++	++	++	++	++
2e	++	++	++	++	++	+
2f	++	++	++	++	++	+
2g	+	+	+	++	++	+
2h	–	–	–	–	–	–
2i	++	++	++	++	++	+
2j	+	+	–	–	+	+
Metr.**	+	+	–	–	++	+
Strept.***	++	++	–	–	++	–

*"–" – diameter of the growth inhibition zone is less than 10 mm; "+" – diameter of the growth inhibition zone is 10-14 mm; "++" – diameter of the growth inhibition zone is 15-20 mm; "+++" – diameter of the growth inhibition zone is more than 20 mm. ** Metr. – Metronidazole, DMSO solution (the concentration is 30 $\mu\text{g/ml}$); *** Strept. – Streptomycin, H_2O solution (the concentration is 30 $\mu\text{g/ml}$).

CONCLUSIONS

1. The synthesis of novel derivatives of 3-amino-5-methyl-2-(alkylthio)-4-oxo-N-aryl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamides has been performed by alkylation of 3-amino-5-methyl-4-oxo-N-aryl-2-thioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-6-carboxamides.

2. Their antimicrobial activity screening has shown that almost all of the compounds obtained appeared to

be more active than the reference drugs against both *Proteus vulgaris* and *Pseudomonas aeruginosa* strains. Compound **2d** – 3-amino-2-(benzylthio)-5-methyl-N-(4-methylphenyl)-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide has been identified as the most active one, which moderately inhibited the growth of all of the test strains and showed the high activity against *Bacillus subtilis* and *Candida albicans* fungi.

REFERENCES

1. Коваленко С.М., Власов С.В., Федосов А.І. та ін. // Вісник фармації. – 2008. – №1. – С. 3-7.
2. Abu-Hashem A.A., Abu-Zied K.M., El-Shehry M.F. // Monatsh. Chem. – 2011. – Vol. 142, №5. – P. 539-545.
3. Alagarsamy V., Meena S., Ramseshu K.V. et al. // Eur. J. Med. Chem. – 2006. – Vol. 41, №11. – P. 1293-1300.
4. Al-Taisan K.M., Al-Hazimi H.M.A., Al-Shihry S.S. // Molecules. – 2010. – Vol. 15, №6. – P. 3932-3957.
5. American Society for Microbiology. Manual of Antimicrobial Susceptibility Testing. – American Society for Microbiology: Washington, 2005. – P. 236.
6. Ashalatha B.V., Narayana B., Vijaya Raj K.K. et al. // Eur. J. Med. Chem. – 2007. – Vol. 42, №5. – P. 719-728.
7. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. Document M100-S22, Vol. 32, №3, CLSI, Wayne, PA, January, 2012.
8. Gaber H.M., Bagley M.C. // Eur. J. Chem. – 2011. – Vol. 2, №2. – P. 214-222.
9. Kovalenko S.M., Vlasov S.V., Silin O.V. et al. // ЖОрФХ. – 2010. – Vol. 8, №1 (29). – P. 20-24.
10. Tkachenko O.V., Vlasov S.V., Kovalenko S.M. et al. // ЖОрФХ. – 2013. – Т. 11, №3 (43). – P. 9-15.

СИНТЕЗ ТА ДОСЛІДЖЕННЯ АНТИМІКРОБНОЇ АКТИВНОСТІ 3-АМІНО-5-МЕТИЛ-2-(АЛКІЛТІО)-4-ОКСО-N-АРИЛ-3,4-ДИГІДРОТІЄНО[2,3-d]ПІРИМІДИН-6-КАРБОКСАМІДІВ
С.В.Власов, С.М.Коваленко, Т.П.Осолодченко, В.П.Черних

Ключові слова: тіофен; піримідин; амід; алкілування

Шляхом алкілування 3-аміно-5-метил-4-оксо-N-арил-2-тіоксо-1,2,3,4-тетрагідротієно[2,3-d]піримідин-6-карбоксамідів заміщеними бензилхлоридами та хлороцтовою кислотою були одержані нові похідні з ряду 3-аміно-5-метил-2-(алкілтіо)-4-оксо-N-арил-3,4-дигідротієно[2,3-d]піримідин-6-карбоксамідів. Структура одержаних сполук була підтверджена даними ¹H ЯМР спектроскопії та елементного аналізу. Для всіх одержаних сполук у спектрах ¹H ЯМР спостерігаються сигнали протонів аміногрупи у вигляді чіткого синглету в діапазоні 5.75-5.84 м.ч., сигнал метильної групи у положенні 5 тієно[2,3-d]піримідинової системи при 2.68-2.75 м.ч. та сигнал протону NH карбоксамідної групи, положення якого варіює від 9.67 м.ч. до 10.61 м.ч. в залежності від природи замісників у ароматичному ядрі амід. Також у спектрах проявляються сигнали протонів груп CH₂ бензильних замісників у діапазоні 4.23-4.29 м.ч., для похідного тіооцтової кислоти цей сигнал знаходиться при 3.82 м.ч. Скринінг антимікробної активності одержаних сполук проводили шляхом дифузії в агар, використовуючи «метод колодязів». Встановлено, що більшість зі сполук виявилась активнішою за препарати порівняння одразу по відношенню до *Proteus vulgaris* та *Pseudomonas aeruginosa*, найбільш активною сполукою виявився 3-аміно-2-(бензилтіо)-5-метил-N-(4-метилфеніл)-4-оксо-3,4-дигідротієно[2,3-d]піримідин-6-карбоксамід, який помірно пригнічував ріст усіх тест-штамів мікроорганізмів та перевищував активність препаратів порівняння Стрептоміцину та Метронідазолу по відношенню до *Bacillus subtilis* та грибів *Candida albicans*.

СИНТЕЗ И ИССЛЕДОВАНИЕ ПРОТИВОМИКРОБНОЙ АКТИВНОСТИ 3-АМИНО-5-МЕТИЛ-2-(АЛКИЛТИО)-4-ОКСО-N-АРИЛ-3,4-ДИГИДРОТИЕНО[2,3-d]ПИРИМИДИН-6-КАРБОКСАМИДОВ

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

Ключевые слова: тиофен; пириимидин; амиды; алкилирование

Путем алкилирования 3-амино-5-метил-4-оксо-N-арил-2-тиоксо-1,2,3,4-тетрагидротиено[2,3-d]пириимидин-6-карбоксамидов замещенными бензилхлоридами и хлоруксусной кислотой были получены новые производные ряда 3-амино-5-метил-2-(алкитио)-4-оксо-N-арил-3,4-дигидротиено[2,3-d]пириимидин-6-карбоксамидов. Структура полученных соединений была подтверждена данными ¹H ЯМР спектроскопии и элементного анализа. Для всех полученных

соединений в спектрах ^1H ЯМР наблюдаются сигналы протонов аминогруппы в виде четкого синглета в диапазоне 5.75-5.84 м.д., сигнал метильной группы в положении 5 тиено[2,3-*d*]пиримидиновой системы при 2.68-2.75 м.д. и сигнал протона NH карбоксамидной группы, положение которого варьирует от 9.67 м.д. до 10.61 м.д. в зависимости от природы заместителей в ароматическом ядре амида. Также в спектрах проявляются сигналы протонов групп CH_2 бензильных заместителей в диапазоне 4.23-4.29 м.д., для производного тиоуксусной кислоты этот сигнал находится при 3.82 м.д. Скрининг противомикробной активности полученных соединений проводили путем диффузии в агар, используя «метод колодцев». Установлено, что большинство соединений оказалось активнее препаратов сравнения сразу по отношению к *Proteus vulgaris* и *Pseudomonas aeruginosa*, наиболее активным соединением является 3-амино-2-(бензилтио)-5-метил-N-(4-метилфенил)-4-оксо-3,4-дигидротиено[2,3-*d*]пиримидин-6-карбоксамид, который умеренно угнетал рост всех тест-штаммов микроорганизмов и превысил активность препаратов сравнения Стрептомицина и Метронидазола по отношению к *Bacillus subtilis* и грибам *Candida albicans*.

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

UDC 547.856'792'292.057:615.281/.282

SYNTHESIS AND MODIFICATION OF 2-[8-R₁-9-R₂-10-R₃-3-R-2-OXO-2H-[1,2,4]TRIAZINO[2,3-c]QUINAZOLINE-6-YL) THIO]ACETIC ACIDS AIMED AT SEARCHING EFFECTIVE SUBSTANCES WITH THE ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

I.S.Nosulenko, O.Yu.Voskoboynik, G.G.Berest, S.I.Kovalenko,
O.M.Kamyshnyi, N.M.Polishchuk

Zaporizhzhia State Medical University

Key words: synthesis; quinazolines; triazines; antibacterial action; antifungal action

*In the present paper 50 new derivatives of 2-[8-R₁-9-R₂-10-R₃-3-R-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazoline-6-yl)thio]acetic acids have been described. It has been shown that alkylation of potassium 8-R₁-9-R₂-10-R₃-3-R-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-thiolates by chloroacetic acid, chloroacetamide, N-R₄-chloroacetamides and chloroacetonitrile yield the corresponding 2-[8-R₁-9-R₂-10-R₃-3-R-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazoline-6-yl)thio]acetic acids, their amides and nitriles. For the corresponding acids and nitriles the alternative synthetic approaches have been developed. Limitations of synthetic approaches concerning the synthesis of the target compounds have been also discussed. Thus, it has been shown that amides of 2-[8-R₁-9-R₂-10-R₃-3-R-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazoline-6-yl)thio]acetic acids derivatives can not be prepared by amonolysis of the corresponding ester due to the low reactivity of the compounds mentioned. It has been also stated that the synthesis of nitriles via dehydration of proper amides with phosphorous-oxychloride in dichlormethane was not successful in all cases. This fact was caused by low yields and problems with isolation of the target compounds from the reaction mixture. The structures of the compounds synthesized have been confirmed by ¹H, ¹³C NMR, LC-MS analysis. The compounds synthesized have been tested for the antimicrobial and antifungal activity using standard test cultures: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 885-653. It has been shown that the compounds synthesized exhibit a high antimicrobial activity against *St. aureus* (compounds 3.3-3.6, 4.3-4.6, 4.7, 4.8, 4.13-4.16; MIC 12.5-25 µg/ml) and *C. albicans* (compounds 4.13, 4.14; MIC 12.5 µg/ml). The "structure-activity" relationship has been discussed.*

Quinazoline derivatives play an important role in the present list of medicines. First of all, the compounds mentioned are known as anticancer (afatinib, vandetanib, lapatinib, trimetrexate, gefitinib, raltitrexed), antifungal (albaconazol), antibacterial (nifurquinazol) drugs, etc. [3]. Some recent publications are devoted to the search of chemotherapeutics among condensed quinazolines, in particular benzimidazo[1,2-c]-, benzthiadiazolimidazo[1,2-c]-, triazolo[1,5-c]-, imidazo, [1,2,4]triazolo[4,3-c]-, triazino[2,3-c]quinazolines [1, 2, 4, 5, 7, 8, 10, 13-18]. Highly effective antimicrobial, antifungal, anticancer agents have been found among the compounds mentioned. Following to the strategy aimed at the directed search of biologically active compounds among S-substituted 3-R-6-thioxo-6,7-dihydro-2H-[1,2,4]triazino[2,3-c]quinazoline-2-ones [6-8, 11, 12], we were interested in chemical modification of the carboxylic group, aryl group in position 3 and introduction of halogen- and alkyl- substituents in positions 8, 9, 10 of the structure of the given compounds and evaluation of their antimicrobial and antifungal activity. The data obtained were used to understand the "structure-activity" relationship.

Experimental Part

1. Chemistry

1.1. General methods

Melting points were determined in open capillary tubes and were uncorrected. The elemental 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. ¹H NMR spectra (500 MHz) and ¹³C 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 an "Agilent 1100 Series" high performance liquid chromatograph (Agilent, Palo Alto, CA, USA) equipped with a diode-matrix and an "Agilent LC/MSD SL" mass-selective detector (atmospheric pressure chemical ionization – APCI). Electron impact mass spectra (EI-MS) were recorded on a Varian 1200 L instrument at 70 eV (Varian, USA). The purity of all compounds was checked by ¹H-NMR and LC-MS.

Substances **1.1-1.19** and **2.1-2.19** were synthesized according to the procedures reported [7, 8]. Other starting

materials and solvents were obtained from commercially available sources and used without additional purification.

1.2. General procedure for the synthesis of [(8- R_1 -9- R_2 -10- R_3 -3- R -2-oxo-2H-[1,2,4]triazino[2,3- c]quinazoline-6-yl)thio]acetic acids (3.1-3.14).

Method A. Add the solution of 0.94 g (10 mmol) chloroacetic acid with 0.40 g (10 mmol) of sodium hydroxide in 5 ml of water to the solution of potassium of the proper 8- R_1 -9- R_2 -10- R_3 -3- R -2-oxo-2H-[1,2,4]triazino[2,3- c]quinazoline-6-thiolates (**2.1-2.18**) (10 mmol) in 20 ml of water, reflux for 1.5 hours to the neutral pH. Then add 50 ml of water to the resulted mixture and filter. Acidify a filtrate with hydrochloric acid to pH 3. Filter the solid obtained and dry. Recrystallize compounds from dioxane.

Method B. Add the proper 8- R_1 -9- R_2 -10- R_3 -3- R -6-thio-6,7-dihydro-2H-[1,2,4]triazino[2,3- c]quinazoline-2-ones (**1.1-1.18**) (5 mmol) and 0.47 g (5 mmol) of chloroacetic acid to the solution of 0.23 g (10 mmol) of metallic sodium in 20 ml of ethanol, reflux for 1.5 hours to the neutral pH. Then add 50 ml of water to the resulted mixture and filter. Acidify a filtrate with hydrochloric acid to pH 3. Filter the solid obtained and dry. Recrystallize compounds from dioxane.

2-[(3-Methyl-2-oxo-2H-[1,2,4]triazino[2,3- c]quinazoline-6-yl)thio]acetic acids (3.1). Yield – 71.9% (method A), 69.4% (method B). M.p. – 238-240°C; ^1H NMR: δ = 2.36 (s, 3H, CH_3), 4.06 (s, 2H, $-\text{SCH}_2$), 7.68-7.59 (m, 2H, H-8, 10), 7.93 (t, 1H, J = 7.9, H-9), 8.41 (d, 1H, J = 7.9, H-11), 12.90 (s, 1H, COOH); ^{13}C NMR: δ = 18.19 (CH_3), 34.21 (SCH_2), 118.50 (11a), 126.00 (8), 126.76 (10), 128.01 (11), 136.02 (9), 144.09 (11b), 151.93 (3), 154.41 (6), 155.28 (7a), 160.98 (2), 170.04 (COOH); LC-MS, m/z = 303 [M+1], 304 [M+2]; Anal. Calcd for $\text{C}_{13}\text{H}_{10}\text{N}_4\text{O}_3\text{S}$: C, 51.65; H, 3.33; N, 18.53; S, 10.61; Found: C, 51.66; H, 3.34; N, 18.52; S, 10.61.

2-[(3-Phenyl-2-oxo-2H-[1,2,4]triazino[2,3- c]quinazoline-6-yl)thio]acetic acids (3.2). Yield – 75.8% (method A), 70.3% (method B). M.p. – 270-272°C; ^1H NMR: δ = 4.14 (s, 2H, $-\text{SCH}_2$), 7.66-7.58 (m, 3H, H-3', 4', 5'), 7.74-7.68 (m, 2H, H-8, 10), 7.98 (t, 1H, J = 7.9, H-9), 8.28 (d, 2H, J = 8.2, H-2', 6'), 8.49 (d, 1H, J = 7.9, H-11), 12.97 (s, 1H, COOH); LC-MS, m/z = 307 [M+1], 309 [M+3]; Anal. Calcd for $\text{C}_{18}\text{H}_{12}\text{N}_4\text{O}_3\text{S}$: C, 59.33; H, 3.32; N, 15.38; S, 8.80; Found: C, 59.33; H, 3.33; N, 15.37; S, 8.81.

2-[(3-(4'-Methylphenyl)-2-oxo-2H-[1,2,4]triazino[2,3- c]quinazoline-6-yl)thio]acetic acids (3.3). Yield – 84.9% (method A) and 80.6% (method B). M.p. – 234-236°C; ^1H NMR: δ = 2.38 (s, 3H, CH_3), 4.10 (s, 2H, $-\text{SCH}_2$), 7.37 (d, 2H, J = 8.2, H-3', 5'), 7.71-7.61 (m, 2H, H-8, 10), 7.94 (t, 1H, J = 7.9, H-9), 8.20 (d, 2H, J = 8.2, H-2', 6'), 8.44 (d, 1H, J = 7.9, H-11), 12.93 (s, 1H, COOH); LC-MS, m/z = 321 [M- CH_2COOH] $^+$, 379 [M+1], 381 [M+3]; Anal. Calcd for $\text{C}_{19}\text{H}_{14}\text{N}_4\text{O}_3\text{S}$: C, 60.31; H, 3.73; N, 14.81; S, 8.47; Found: C, 60.32; H, 3.73; N, 14.81; S, 8.49.

2-[(3-(3',4'-Dimethylphenyl)-2-oxo-2H-[1,2,4]triazino[2,3- c]quinazoline-6-yl)thio]acetic acids (3.4). Yield – 85.4% (method A), 80.9% (method B). M.p. – 226-228°C; ^1H NMR: δ = 2.31 (d, 6H, J = 4.1, 3,4-(CH_3) $_2$), 4.11 (s, 2H, $-\text{SCH}_2$), 7.33 (d, 1H, J = 8.1, H-5'), 7.72-7.64 (m, 2H, H-10, 8), 7.95 (t, 1H,

J = 7.9, H-9), 8.04 (d, 1H, J = 8.1, H-6'), 8.07 (s, 1H, H-2'), 8.46 (d, 1H, J = 7.9, H-11), 12.99 (s, 1H, COOH); LC-MS, m/z = 335 [M- CH_2COOH] $^+$, 393 [M+1], 395 [M+3]; Anal. Calcd for $\text{C}_{20}\text{H}_{16}\text{N}_4\text{O}_3\text{S}$: C, 61.21; H, 4.11; N, 14.28; S, 8.17; Found: C, 61.23; H, 4.13; N, 14.29; S, 8.18.

2-[(3-(4'-Ethylphenyl)-2-oxo-2H-[1,2,4]triazino[2,3- c]quinazoline-6-yl)thio]acetic acids (3.5). Yield – 75.9%. M.p. – 161-163°C; ^1H NMR δ : 1.31 (t, J = 7.5 Hz, 3H, CH_2CH_3), 2.77 (q, J = 7.3 Hz, 2H, CH_2CH_3), 4.07 (s, 2H, $-\text{SCH}_2$), 7.37 (d, J = 8.2 Hz, 2H, 3-Ph H-3', 5'), 7.65 (t, J = 7.6 Hz, 1H, H-10), 7.75 (d, J = 8.0 Hz, 1H, H-8), 7.93 (t, J = 7.5 Hz, 1H, H-9), 8.28 (d, J = 8.0 Hz, 2H, 3-Ph H-2', 6'), 8.53 (d, J = 7.9 Hz, 1H, H-11), 12.79 (s, 1H, COOH); LC-MS, m/z = 393 [M+1], 395 [M+3]; Anal. calcd. for $\text{C}_{20}\text{H}_{16}\text{N}_4\text{O}_3\text{S}$: C, 61.21; H, 4.11; N, 14.28; S, 8.17; Found: C, 61.24; H, 4.11; N, 14.27; S, 8.19.

2-[(3-(4'-*i*-Propylphenyl)-2-oxo-2H-[1,2,4]triazino[2,3- c]quinazoline-6-yl)thio]acetic acids (3.6). Yield – 43.3%. M.p. – 236-238°C; ^1H NMR δ : 1.32 (d, J = 6.8 Hz, 6H, $\text{CH}(\text{CH}_3)_2$), 3.02 (dt, J = 13.3, 6.5 Hz, 1H, $\text{CH}(\text{CH}_3)_2$), 4.05 (s, 2H, $-\text{SCH}_2$), 7.38 (d, J = 8.1 Hz, 2H, 3-Ph H-3', 5'), 7.63 (t, J = 7.5 Hz, 1H, H-10), 7.75 (d, J = 8.0 Hz, 1H, H-8), 7.91 (t, J = 7.5 Hz, 1H, H-9), 8.29 (d, J = 8.1 Hz, 2H, 3-Ph H-2', 6'), 8.54 (d, J = 7.9 Hz, 1H, H-11), 12.82 (s, 1H, COOH); LC-MS, m/z = 407 [M+1], 409 [M+3]; Anal. calcd. for $\text{C}_{21}\text{H}_{18}\text{N}_4\text{O}_3\text{S}$: C, 62.05; H, 4.46; N, 13.78; S, 7.89; Found: C, 62.07; H, 4.45; N, 13.79; S, 7.88.

2-2[(3-(4'-Methoxyphenyl)-2-oxo-2H-[1,2,4]triazino[2,3- c]quinazoline-6-yl)thio]acetic acids (3.7). Yield – 77.4% (method A), 70.3% (method B). M.p. – 238-242°C; ^1H NMR: δ = 3.84 (s, 3H, OCH_3), 4.10 (s, 2H, $-\text{SCH}_2$), 7.11 (d, 2H, J = 8.8, H-3', 5'), 7.72-7.60 (m, 2H, H-10, 8), 7.93 (t, 1H, J = 7.9, H-9), 8.34 (d, 2H, J = 8.8, H-2', 6'), 8.44 (d, 1H, J = 7.9, H-11), 12.89 (s, 1H, COOH); ^{13}C NMR: δ = 34.22 (SCH_2), 55.94 (OCH_3), 114.45 (3', 5'-Ph), 118.21 (11a), 124.27 (8), 126.00 (10), 126.84 (1'-Ph), 128.04 (11), 131.70 (2', 6'-Ph), 135.90 (9), 144.04 (11b), 148.79 (3), 150.66 (6), 154.63 (7a), 160.20 (2), 162.55 (4'-Ph), 170.08 (COOH); LC-MS, m/z = 395 [M+1], 397 [M+3]; Anal. Calcd for $\text{C}_{19}\text{H}_{14}\text{N}_4\text{O}_4\text{S}$: C, 57.86; H, 3.58; N, 14.21; S, 8.13; Found: C, 57.85; H, 3.54; N, 14.20; S, 8.12.

2-[(3-(4'-Ethoxyphenyl)-2-oxo-2H-[1,2,4]triazino[2,3- c]quinazoline-6-yl)thio]acetic acids (3.8). Yield – 83.6%. M.p. – 245-247°C; LC-MS, m/z = 409 [M+1], 411 [M+3]; Anal. calcd. for $\text{C}_{20}\text{H}_{16}\text{N}_4\text{O}_4\text{S}$: C, 58.81; H, 3.95; N, 13.72; S, 7.85; Found: C, 58.80; H, 3.95; N, 13.74; S, 7.87.

2-[(3-(4'-Fluorophenyl)-2-oxo-2H-[1,2,4]triazino[2,3- c]quinazoline-6-yl)thio]acetic acids (3.9). Yield – 76.2%. M.p. – 230-232°C; ^1H NMR δ : 4.09 (s, 2H, $-\text{SCH}_2$), 9.46-6.62 (m, 8H, H-8, 9, 10, 11, 3-Ph H-2', 3', 5', 6'), 12.83 (s, 1H, COOH); LC-MS, m/z = 383 [M+1], 385 [M+3]; Anal. calcd. for $\text{C}_{18}\text{H}_{11}\text{FN}_4\text{O}_3\text{S}$: C, 56.54; H, 2.90; N, 14.65; S, 8.39; Found: C, 56.57; H, 2.90; N, 14.64; S, 8.38.

2-[(8-Methyl-3-phenyl)-2-oxo-2H-[1,2,4]triazino[2,3- c]quinazoline-6-yl)thio]acetic acids (3.10). Yield – 78.5%. M.p. – 255-257°C; ^1H NMR δ : 2.65 (s, 3H, CH_3), 4.05 (s, 2H, $-\text{SCH}_2$), 7.66-7.41 (m, 4H, H-10, 3-Ph H-3', 4', 5'), 7.76 (d, J = 6.9 Hz, 1H, H-9), 8.46-8.25 (m, 3H, H-11, 3-Ph H-2', 6'), 12.72 (s, 1H, COOH); LC-MS, m/z = 379 [M+1], 381 [M+3]; Anal. calcd. for $\text{C}_{19}\text{H}_{14}\text{N}_4\text{O}_3\text{S}$: C, 60.31; H, 3.73; N, 14.81; S, 8.47; Found: C, 60.34; H, 3.73; N, 14.80; S, 8.45.

2-[(9-Fluoro-3-phenyl)-2-oxo-2H-[1,2,4]triazino[2,3- c]quinazoline-6-yl)thio]acetic acids (3.11). Yield –

75.9%. M.p. – 238–240°C; $^1\text{H NMR}$ δ : 4.08 (s, 2H, $-\text{SCH}_2$), 7.68–7.39 (m, 5H, H-8, 10, 3-Ph H-3', 4', 5'), 8.32 (d, $J = 7.0$ Hz, 2H, 3-Ph H-2', 6'), 8.60 (dd, $J = 8.40, 6.10$ Hz, 1H, H-11), 12.94 (s, 1H, COOH); LC-MS, $m/z = 383$ [M+1], 385 [M+3]; Anal. calcd. for $\text{C}_{18}\text{H}_{11}\text{FN}_4\text{O}_3\text{S}$: C, 56.54; H, 2.90; N, 14.65; S, 8.39; Found: C, 56.51; H, 2.90; N, 14.64; S, 8.41.

2-[(9-Fluoro-3-(4'-fluorophenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazoline-6-yl)thio]acetic acids (3.12). Yield – 50.6%. M.p. – 247–249°C; $^1\text{H NMR}$ δ : 4.08 (s, 2H, $-\text{SCH}_2$), 7.29 (t, 2H, 3-Ph H-3', 5'), 7.47–7.40 (m, 2H, H-8, 10), 8.42 (t, 2H, 3-Ph H-2', 6'), 8.48 (t, 1H, H-11); LC-MS, $m/z = 401$ [M+1], 403 [M+3]; Anal. calcd. for $\text{C}_{18}\text{H}_{10}\text{F}_2\text{N}_4\text{O}_3\text{S}$: C, 54.00; H, 2.52; N, 13.99; S, 8.01; Found: C, 54.03; H, 2.52; N, 13.98; S, 8.03.

2-[(10-Chloro-3-phenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazoline-6-yl)thio]acetic acids (3.13). Yield – 64.6%. M.p. – 258–260°C; $^1\text{H NMR}$ δ : 4.07 (s, 2H, $-\text{SCH}_2$), 7.65–7.39 (m, 3H, 3-Ph H-3', 4', 5'), 7.76 (d, $J = 8.2$ Hz, 1H, H-8), 7.91 (d, $J = 8.2$ Hz, 1H, H-9), 8.50–8.22 (m, 3H, H-11, 3-Ph H-2', 6'), 13.63 (s, 1H, COOH); LC-MS, $m/z = 399$ [M+1], 401 [M+3], 402 [M+4]; Anal. calcd. for $\text{C}_{18}\text{H}_{11}\text{ClN}_4\text{O}_3\text{S}$: C, 54.21; H, 2.78; N, 14.05; S, 8.04; Found: C, 54.24; H, 2.78; N, 14.03; S, 8.02.

2-[(10-Bromo-3-(4'-fluorophenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazoline-6-yl)thio]acetic acids (3.14). Yield – 70.5%. M.p. – 257–259°C; LC-MS, $m/z = 461$ [M]⁺, 465 [M+4]; Anal. calcd. for $\text{C}_{18}\text{H}_{10}\text{BrFN}_4\text{O}_3\text{S}$: C, 46.87; H, 2.19; N, 12.15; S, 6.95; Found: C, 46.88; H, 2.19; N, 12.16; S, 6.93.

1.3. General procedure for the synthesis of $N\text{-R}_1\text{-}[(8\text{-R}_1\text{-}9\text{-R}_2\text{-}10\text{-R}_3\text{-}3\text{-R-}2\text{-oxo-}2\text{H-[1,2,4]triazino[2,3-c]quinazoline-6-yl)thio]acetamides$ (4.1–4.26). Add 0.93 g (10 mmol) of chloracetamide or 10 mmol $N\text{-R}_4\text{-chloracetamides}$ to the suspension of potassium of the proper $8\text{-R}_1\text{-}9\text{-R}_2\text{-}10\text{-R}_3\text{-}3\text{-R-}2\text{-oxo-}2\text{H-[1,2,4]triazino[2,3-c]quinazoline-6-thiolates}$ (2.1–2.18) (10 mmol) in 20 ml of propanol-2 and reflux for 1–1.5 hours. Cool the resulted mixture, filter the solid and dry. Crystallize the compounds obtained from dioxane–water mixture.

2-[(3-Methyl-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetamide (4.1). Yield – 87.3%. M.p. – 289–291°C; $^1\text{H NMR}$ δ : 2.45 (s, 3H, CH_3), 3.93 (s, 2H, $-\text{SCH}_2$), 7.08 (s, 1H, NH_2), 7.50 (s, 1H, NH_2), 7.62 (t, $J = 8.2$ Hz, 1H, H-10), 7.77 (d, $J = 8.2$ Hz, 1H, H-8), 7.91 (t, $J = 7.3$ Hz, 1H, H-9), 8.52 (d, $J = 7.5$ Hz, 1H, H-11); LC-MS, $m/z = 302$ [M+1], 304 [M+3]; Anal. calcd. for $\text{C}_{13}\text{H}_{11}\text{N}_5\text{O}_2\text{S}$: C, 51.82; H, 3.68; N, 23.24; S, 10.64; Found: C, 51.82; H, 3.69; N, 23.24; S, 10.63.

2-[(3-Phenyl-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetamide (4.2). Yield – 82.3%. M.p. – 285–288°C; $^1\text{H NMR}$ δ : 3.98 (s, 2H, $-\text{SCH}_2$), 7.12 (s, 1H, NH_2), 7.61–7.43 (m, 4H, 3-Ph 3,5, NH_2), 7.66 (t, $J = 7.6$ Hz, 1H, H-10), 7.80 (d, $J = 7.7$ Hz, 1H, H-8), 7.94 (t, $J = 7.1$ Hz, 1H, H-9), 8.36 (d, $J = 7.2$ Hz, 2H, 3-Ph H-2,6), 8.56 (d, $J = 7.6$ Hz, 1H, H-11); LC-MS, $m/z = 366$ [M+1]; Anal. calcd. for $\text{C}_{18}\text{H}_{13}\text{N}_5\text{O}_2\text{S}$: C, 59.49; H, 3.61; N, 19.27; S, 8.82; Found: 59.48; H, 3.61; N, 19.27; S, 8.83.

2-[(3-(4-Methylphenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetamide (4.3). Yield – 69.3%. M.p. – 264–267°C; IR (cm^{-1}): 3434, 3314, 1682, 1664, 1589, 1561, 1543, 1496, 1469, 1400, 1367, 1341, 1311, 1272, 1240, 1190, 1161, 1135, 1108, 1075, 1021, 991, 940, 885, 833, 784,

772, 707, 686, 643, 629; $^1\text{H NMR}$ δ : 2.39 (s, 3H, CH_3), 4.00 (s, 2H, $-\text{SCH}_2$), 7.30 (s, 1H, $-\text{C}(\text{O})\text{NH}_2$), 7.39 (d, 2H, $J = 8.2$, H-3', 5' Ph), 7.78–7.64 (m, 3H, H-8, 10, $-\text{C}(\text{O})\text{NH}_2$), 7.96 (t, 1H, $J = 7.9$, H-9), 8.22 (d, 2H, $J = 8.2$, H-2', 6' Ph), 8.46 (d, 1H, $J = 7.9$, H-11); LC-MS, $m/z = 378$ [M+1], 380 [M+3]; Anal. calcd. for $\text{C}_{19}\text{H}_{15}\text{N}_5\text{O}_2\text{S}$: C, 60.47; H, 4.01; N, 18.56; S, 8.50; Found: C, 59.47; H, 4.03; N, 18.55; S, 8.52.

2-[(3-(3,4-Dimethylphenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetamide (4.4). Yield – 94.05%. M.p. – 272–275°C; $^1\text{H NMR}$ δ : 2.33 (s, 3H, 3- CH_3), 2.37 (s, 3H, 4- CH_3), 3.95 (s, 2H, $-\text{SCH}_2$), 7.07 (s, 1H, NH_2), 7.51 (s, 1H, NH_2), 7.28 (d, $J = 7.9$ Hz, 1H, 3-Ph H-5'), 7.63 (t, $J = 8.2$ Hz, 1H, H-10), 7.76 (d, $J = 8.2$ Hz, 1H, H-8), 7.90 (t, $J = 7.3$ Hz, 1H, H-9), 8.03 (d, $J = 7.8$ Hz, 1H, 3-Ph H-6'), 8.12 (s, 1H, 3-Ph H-2'), 8.53 (d, $J = 7.5$ Hz, 1H, H-11); LC-MS, $m/z = 392$ [M+1], 394 [M+4]; Anal. calcd. for $\text{C}_{20}\text{H}_{17}\text{N}_5\text{O}_2\text{S}$: C, 61.37; H, 4.38; N, 17.89; S, 8.19; Found: C, 61.36; H, 4.38; N, 17.89; S, 8.20.

2-[(3-(4'-Ethylphenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetamide (4.5). Yield – 86.4%. M.p. – 233–235°C; $^1\text{H NMR}$ δ : 1.30 (t, $J = 7.4$ Hz, 3H CH_3), 2.75 (dd, $J = 14.8, 7.2$ Hz, 2H, CH_2CH_3), 3.97 (s, 2H, $-\text{SCH}_2$), 7.13 (s, 1H, NH_2), 7.35 (d, $J = 8.0$ Hz, 2H, 3-Ph H-3, 5), 7.54 (s, 1H, NH_2), 7.63 (t, $J = 7.5$ Hz, 1H, H-10), 7.78 (d, $J = 8.1$ Hz, 1H, H-8), 7.91 (t, $J = 7.3$ Hz, 1H, H-9), 8.29 (d, $J = 8.0$ Hz, 2H, 3-Ph H-2,6), 8.53 (d, $J = 7.8$ Hz, 1H, H-11); LC-MS, $m/z = 392$ [M+1], 394 [M+3]; Anal. calcd. for $\text{C}_{20}\text{H}_{17}\text{N}_5\text{O}_2\text{S}$: C, 61.37; H, 4.38; N, 17.89; S, 8.19; Found: C, 61.39; H, 4.38; N, 17.88; S, 8.18.

2-[(3-(4'-*tert*-Butylphenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetamide (4.6). Yield – 79.5%. M.p. – 265–268°C; $^1\text{H NMR}$ δ : 1.39 (s, 9H, $\text{C}(\text{CH}_3)_3$), 3.97 (s, 2H, $-\text{SCH}_2$), 7.13 (s, 1H, NH_2), 7.54 (d, $J = 7.9$ Hz, 3H, 3-Ph H-3,5, NH_2), 7.62 (t, $J = 7.4$ Hz, 1H, H-10), 7.77 (d, $J = 8.0$ Hz, 1H, H-8), 7.90 (t, $J = 7.3$ Hz, 1H, H-9), 8.30 (d, $J = 8.2$ Hz, 2H, 3-Ph H-2,6), 8.53 (d, $J = 7.8$ Hz, 1H, H-11); LC-MS, $m/z = 420$ [M+1], 422 [M+3]; Anal. calcd. for $\text{C}_{22}\text{H}_{21}\text{N}_5\text{O}_2\text{S}$: C, 62.99; H, 5.05; N, 16.69; S, 7.64; Found: C, 62.99; H, 5.06; N, 16.69; S, 7.63.

2-[(3-(4'-Methoxyphenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetamide (4.7). Yield – 88.9%. M.p. – 253–255°C; $^1\text{H NMR}$ δ : 3.90 (s, 3H, CH_3), 3.97 (s, 2H, $-\text{SCH}_2$), 7.07 (d, $J = 8.2$ Hz, 2H, 3-Ph H-3', 5'), 7.13 (s, 1H, NH_2), 7.55 (s, 1H, NH_2), 7.64 (t, $J = 7.5$ Hz, 1H, H-10), 7.79 (d, $J = 7.6$ Hz, 1H, H-8), 7.92 (t, $J = 7.5$ Hz, 1H, H-9), 8.42 (d, $J = 8.2$ Hz, 2H, 3-Ph H-2', 6'), 8.54 (d, $J = 7.1$ Hz, 1H, H-11); LC-MS, $m/z = 394$ [M+1], 396 [M+3]; Anal. calcd. for $\text{C}_{19}\text{H}_{15}\text{N}_5\text{O}_3\text{S}$: C, 58.01; H, 3.84; N, 17.80; S, 8.15; Found: C, 58.00; H, 3.84; N, 17.80; S, 8.16.

2-[(3-(4'-Ethoxyphenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetamide (4.8). Yield – 91.6%. M.p. – 288–291°C; $^1\text{H NMR}$ δ : 1.44 (t, 3H, CH_3), 3.96 (s, 2H, $-\text{SCH}_2$), 4.14 (q, $J = 6.3$ Hz, 2H, OCH_2CH_3), 6.99 (d, $J = 8.0$ Hz, 2H, 3-Ph H-3', 5'), 7.14 (s, 1H, NH_2), 7.53 (s, 1H, NH_2), 7.64 (t, $J = 7.6$ Hz, 1H, H-10), 7.80 (d, $J = 7.6$ Hz, 1H, H-8), 7.93 (t, $J = 7.6$ Hz, 1H, H-9), 8.42 (d, $J = 8.0$ Hz, 2H, 3-Ph H-2', 6'), 8.53 (d, $J = 7.6$ Hz, 1H, H-11); Anal. calcd. for $\text{C}_{20}\text{H}_{17}\text{N}_5\text{O}_3\text{S}$: C, 58.96; H, 4.21; N, 17.19; S, 7.87; Found: C, 58.96; H, 4.22; N, 17.19; S, 7.86.

N -(3-Fluorophenyl)-2-[(3-(4'-methoxyphenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetamide (4.9). Yield – 87.8%. M.p. – 274–276°C; $^1\text{H NMR}$ δ : 3.91

(s, 3H, -OCH₃), 4.22 (s, 2H, -SCH₂), 6.78 (dd, *J* = 10.7, 8.3 Hz, 1H, 6-Ph H-4), 7.08 (d, *J* = 8.8 Hz, 2H, 3-Ph H-3', 5'), 7.44-7.22 (m, 2H, 6-Ph H-2, 6), 7.69 – 7.54 (m, 2H, H-10, 6-Ph H-5), 7.74 (d, *J* = 8.8 Hz, 1H, H-8), 7.91 (t, *J* = 7.8, 1H, H-9), 8.44 (d, *J* = 8.2 Hz, 2H, 3-Ph H-2', 6'), 8.54 (d, *J* = 7.8 Hz, 1H, H-11), 10.53 (s, 1H, NH); LC-MS, *m/z* = 488 [M+1], 490 [M+3]; Anal. calcd. for C₂₅H₁₈FN₃O₃S: C, 61.59; H, 3.72; N, 14.37; S, 6.58; Found: C, 61.61; H, 3.72; N, 14.36; S, 6.57.

2-[(3-(4'-Fluorophenyl)-2-oxo-2H-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.10). Yield – 81.1%. M.p. – 284-287°C; ¹H NMR δ: 3.95 (s, 2H, -SCH₂), 7.12 (s, 1H, NH₂), 7.29 (t, *J* = 8.5 Hz, 2H, 3-Ph H-3', 5'), 7.55 (s, 1H, NH₂), 7.71 (t, *J* = 7.4 Hz, 1H, H-10), 7.89 (d, *J* = 8.0 Hz, 1H, H-8), 7.99 (t, *J* = 7.3 Hz, 1H, H-9), 8.43 (dd, *J* = 7.5, 5.9 Hz, 2H, 3-Ph H-2', 6'), 8.59 (d, *J* = 7.9 Hz, 1H, H-11); LC-MS, *m/z* = 382 [M+1], 384 [M+3]; Anal. calcd. for C₁₈H₁₂FN₅O₂S: C, 56.69; H, 3.17; 18.36; S, 8.41; Found: C, 56.68; H, 3.17; 18.376; S, 8.41.

2-[(8-Methyl-3-phenyl-2-oxo-2H-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.11). Yield – 86.2%. M.p. – 282-284°C; ¹H NMR δ: 2.75 (s, 3H, CH₃), 3.98 (s, 2H, -SCH₂), 7.15 (s, 1H, NH₂), 7.62-7.43 (m, 5H, H-10, 3-Ph H-3', 4', 5', NH₂), 7.82 (d, *J* = 5.6 Hz, 1H, H-9), 8.30 (d, *J* = 8.8 Hz, 2H, 3-Ph H-2', 6'), 8.43 (d, *J* = 7.8 Hz, 1H, H-11); Anal. calcd. for C₁₉H₁₅N₅O₂S: C, 60.46; H, 4.01; N, 18.56; S, 8.50; Found: C, 60.45; H, 4.01; N, 18.56; S, 8.51.

2-[(8-Bromo-3-(4-fluorophenyl)-2-oxo-2H-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.12). Yield – 79.5%. M.p. – 270-273°C; ¹H NMR δ: 3.94 (s, 2H, -SCH₂), 6.60 (t, *J* = 7.6 Hz, 1H, H-10), 7.15 (s, 1H, NH₂), 7.21 (t, *J* = 7.8 Hz, 2H, 3-Ph H-3', 5'), 7.54 (s, 1H, NH₂), 7.66 (d, *J* = 7.4 Hz, 1H, H-9), 7.75 (d, *J* = 7.7 Hz, 1H, H-11), 8.33 (t, 2H, *J* = 5.3 Hz, 3-Ph H-2', 6'); LC-MS, *m/z* = 460 [M]; Anal. calcd. for C₁₈H₁₁BrFN₅O₂S: C, 46.97; H, 2.41; N, 15.22; S, 6.97; Found: C, 46.96; H, 2.41; N, 15.22; S, 6.98.

2-[(9-Fluoro-3-phenyl-2-oxo-2H-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.13). Yield – 91.2%. M.p. – 286-289°C; ¹H NMR δ: 3.95 (s, 2H, -SCH₂), 7.13 (s, 1H, NH₂), 7.47-7.36 (t, 1H, H-10), 7.54 (m, 5H, H-8, 3-Ph H-3', 4', 5', NH₂), 8.33 (d, *J* = 5.7 Hz, 2H, 3-Ph H-2', 6'), 8.59 (dd, *J* = 6.2, 4.6 Hz, 1H, H-11); LC-MS, *m/z* = 382 [M+1], 384 [M+3]; Anal. calcd. for C₁₈H₁₂FN₅O₂S: C, 56.69; H, 3.17; N, 18.36; S, 8.41; Found: C, 56.69; H, 3.16; N, 18.36; S, 8.42.

2-[(9-Fluoro-3-(4'-methoxyphenyl)-2-oxo-2H-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.14). Yield – 89.1%. M.p. – 251-254°C; ¹H NMR δ: 3.89 (s, 3H, -OCH₃), 3.95 (s, 2H, -SCH₂), 7.05 (d, *J* = 8.6 Hz, 2H, 3-Ph H-3', 5'), 7.14 (s, 1H, NH₂), 7.43 (dd, *J* = 8.6, 6.5 Hz, 1H, H-10), 7.51 (d, *J* = 8.7 Hz, 1H, H-8), 7.56 (s, 1H, NH₂), 8.40 (d, *J* = 8.7 Hz, 2H, 3-Ph H-2,6), 8.59 (dd, *J* = 8.0, 5.4 Hz, 1H, H-11); LC-MS, *m/z* = 412 [M+1], 414 [M+3]; Anal. calcd. for C₁₉H₁₄FN₅O₃S: C, 55.47; H, 3.43; N, 17.02; S, 7.79; Found: C, 55.43; H, 3.46; N, 17.01; S, 7.80.

N-(4-Bromophenyl)-2-[(9-fluoro-3-(4-methoxyphenyl)-2-oxo-2H-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.15). Yield – 92.6%. M.p. – 304-306°C; ¹H NMR δ: 3.90 (s, 3H, -OCH₃), 4.20 (s, 2H, -SCH₂), 7.08 (d, *J* = 8.7 Hz, 2H, 3-Ph H-3', 5'), 7.51-7.32 (m, 4H, H-8, 10, 6-Ph H-2', 6'), 7.62 (d, *J* = 8.5 Hz, 2H, 6-Ph H-3', 5'), 8.41 (d, *J* = 8.7 Hz, 2H, 3-Ph H-2', 6'), 8.57 (dd, *J* = 8.4, 6.3 Hz, 1H, H-11), 10.46 (s, 1H, NH); Anal. calcd. for C₂₅H₁₇BrFN₅O₃S: C, 53.01;

H, 3.03; Br, 14.11; F, 3.35; N, 12.36; S, 5.66; Found: C, 53.04; H, 3.03; Br, 14.11; F, 3.35; N, 12.34; S, 5.65.

2-[(9-Fluoro-3-(4-fluorophenyl)-2-oxo-2H-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.16). Yield – 71.8%. M.p. – 274-276°C; ¹H NMR δ: 3.96 (s, 2H, -SCH₂), 7.17 (s, 1H, NH₂), 7.28 (t, *J* = 8.4 Hz, 2H, 3-Ph H-3', 5'), 7.53 (t, *J* = 7.5 Hz, 1H, H-10), 7.55 (s, 1H, NH₂), 7.56 (d, *J* = 9.2 Hz, 1H, H-8), 8.39 (t, *J* = 5.7 Hz, 2H, 3-Ph H-2', 6'), 8.65 (dd, *J* = 8.3, 6.0 Hz, 1H, H-11); LC-MS, *m/z* = 400 [M+1], 402 [M+3]; Anal. calcd. for C₁₈H₁₁F₂N₅O₂S: C, 54.13; H, 2.78; N, 17.54; S, 8.03; Found: C, 54.12; H, 2.78; N, 17.55; S, 8.03.

2-[(9-Bromo-3-(4-fluorophenyl)-2-oxo-2H-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.17). Yield – 84.62%. M.p. – 282-285°C; ¹H NMR δ: 3.95 (s, 2H, -SCH₂), 7.12 (s, 1H, NH₂), 7.30 (t, *J* = 8.4 Hz, 2H, 3-Ph H-3,5), 7.75 (d, *J* = 8.1 Hz, 1H, H-10), 7.55 (s, 1H, NH₂), 8.00 (s, 1H, H-8), 8.55-8.32 (m, 3H, H-11, 3-Ph H-2,6); LC-MS, *m/z* = 461 [M+1]; Anal. calcd. for C₁₈H₁₁BrFN₅O₂S: C, 46.97; H, 2.41; N, 15.22; S, 6.97; Found: C, 46.97; H, 2.42; N, 15.22; S, 6.96.

N-(4-(Trifluoromethyl)benzyl)-2-[(9-bromo-3-(4-fluorophenyl)-2-oxo-2H-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.18). Yield – 81.9%. M.p. – 273-276°C; ¹H NMR δ: 4.04 (s, 2H, -SCH₂), 4.44 (d, *J* = 4.6 Hz, 2H, -CH₂), 7.33 (t, *J* = 7.7 Hz, 2H, 3-Ph H-3', 5'), 7.57-7.41 (m, 4H, 6-Ph H-2', 3', 5', 6'), 7.79 (d, *J* = 8.4 Hz, 1H, H-10), 7.90 (s, 1H, H-8), 8.51-8.33 (m, 3H, H-11, 3-Ph H-2', 6'), 8.80 (t, *J* = 5.9 Hz, 1H, NHCO); Anal. calcd. for C₂₆H₁₆BrF₄N₅O₂S: C, 50.50; H, 2.61; Br, 12.92; F, 12.29; N, 11.32; S, 5.19; Found: C, 50.53; H, 2.61; Br, 12.92; F, 12.29; N, 11.31; S, 5.17.

2-[(10-Chloro-3-phenyl-2-oxo-2H-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.19). Yield – 65.9%. M.p. – 273-276°C; ¹H NMR δ: 3.97 (s, 2H, -SCH₂), 7.13 (s, 1H, NH₂), 7.66-7.39 (m, 3H, 3-Ph H-3', 5', NH₂), 7.81 (d, *J* = 8.7 Hz, 1H, H-8), 7.89 (d, *J* = 8.7 Hz, 1H, H-9), 8.36 (d, *J* = 7.1 Hz, 2H, 3-Ph H-2', 6'), 8.47 (s, 1H, H-11); LC-MS, *m/z* = 398 [M+1], 400 [M+3], 401 [M+4]; Anal. calcd. for C₁₈H₁₂ClN₅O₂S: C, 54.34; H, 3.04; N, 17.60; S, 8.06; Found: C, 54.36; H, 3.04; N, 17.59; S, 8.05.

N-(4-Methoxybenzyl)-2-[(10-chloro-2-oxo-3-phenyl-2H-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.20). Yield – 81.4%. M.p. – 242-244°C; ¹H NMR δ: 3.74 (s, 3H, OCH₃), 4.01 (s, 2H, -SCH₂), 4.27 (d, *J* = 5.5 Hz, 2H, -CH₂), 6.74 (d, *J* = 8.2 Hz, 2H, 6-Ph H-3', 5'), 7.17 (d, *J* = 8.1 Hz, 2H, 6-Ph H-2', 6'), 7.66-7.46 (m, 4H, H-8, 3-Ph H-3', 4', 5'), 7.86 (d, *J* = 8.6 Hz, 1H, H-9), 8.35 (d, *J* = 7.3 Hz, 2H, 3-Ph H-2', 6'), 8.45 (s, 1H, H-11), 8.60 (t, *J* = 5.5 Hz, 1H, NH); LC-MS, *m/z* = 519 [M+1], 522 [M+4]; Anal. calcd. for C₂₆H₂₀ClN₅O₃S: C, 60.29; H, 3.89; Cl, 6.84; N, 13.52; S, 6.19; Found: C, 60.32; H, 3.89; Cl, 6.84; N, 13.50; S, 6.22.

N-(4-(Trifluoromethyl)benzyl)-2-[(10-chloro-3-(4-methoxyphenyl)-2-oxo-2H-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.21). Yield – 93.5%. M.p. – 279-282°C; ¹H NMR δ: 3.91 (s, 3H, -OCH₃), 4.05 (s, 2H, -SCH₂), 4.43 (d, *J* = 3.8 Hz, 2H, -CH₂), 7.08 (d, *J* = 7.6 Hz, 2H, 3-Ph H-3', 5'), 7.54-7.40 (m, 5H, 6-Ph H-2', 3', 4', 5', 6'), 7.64 (d, *J* = 7.8 Hz, 1H, H-8), 7.84 (d, *J* = 7.9 Hz, 1H, H-9), 8.51-8.33 (m, 3H, H-11, 3-Ph H-2', 6'), 8.82 (t, *J* = 3.8 Hz, 1H, NH); Anal. calcd. for C₂₇H₁₉ClF₃N₅O₃S: C, 55.34; H, 3.27; Cl, 6.05; F, 9.73; N, 11.95; S, 5.47; Found: C, 55.36; H, 3.27; Cl, 6.05; F, 9.73; N, 11.94; S, 5.46.

2-[(10-Bromo-3-phenyl-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.23). Yield – 64.0%. M.p. – 250-253°C; ¹H NMR δ: 3.97 (s, 2H, -SCH₂), 7.13 (s, 1H, NH₂), 7.65-7.46 (m, 4H, 3-Ph H-3', 4', 5', NH₂), 7.74 (d, *J* = 7.9 Hz, 1H, H-8), 8.02 (d, *J* = 7.8 Hz, 1H, H-9), 8.36 (d, *J* = 6.9 Hz, 2H, 3-Ph H-2', 6'), 8.63 (s, 1H, H-11); LC-MS, *m/z* = 444 [M+2], 446 [M+4]; Anal. calcd. for C₁₈H₁₂BrN₅O₂S: C, 48.88; H, 2.73; N, 15.83; S, 7.25; Found: C, 48.87; H, 2.74; N, 15.83; S, 7.25.

2-[(10-Bromo-3-(4-methylphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.23). Yield – 79.5%. M.p. – 275-278°C; ¹H NMR δ: 2.45 (s, 3H, -CH₃), 3.96 (s, 2H, -SCH₂), 6.32 (d, *J* = 7.9 Hz, 2H, 3-Ph H-3', 5'), 7.13 (s, 1H, NH₂), 7.57 (s, 1H, NH₂), 7.81 (d, *J* = 8.6 Hz, 1H, H-8), 8.07 (dd, *J* = 8.6, 1.6 Hz, 1H, H-9), 8.23 (d, *J* = 8.0 Hz, 2H, 3-Ph H-2', 6'), 8.64 (s, 1H, H-11); LC-MS, *m/z* = 457 [M+1]; Anal. calcd. for C₁₉H₁₄BrN₅O₂S: C, 50.01; H, 3.09; N, 15.35; S, 7.03; Found: C, 50.02; H, 3.09; N, 15.36; S, 7.03.

N-(2-Fluorophenyl)-2-[(10-bromo-3-(4'-methoxyphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.24). Yield – 87.7%. M.p. – 284-286°C; ¹H NMR δ: 3.91 (s, 3H, -OCH₃), 4.26 (s, 2H, -SCH₂), 7.27-7.08 (m, 5H, 3-Ph H-3', 5', 6-Ph H-3', 4', 5'), 7.70 (d, *J* = 8.8 Hz, 1H, 6-Ph H-6'), 8.10-7.88 (m, 2H, H-8, 9), 8.43 (d, *J* = 8.7 Hz, 2H, 3-Ph H-2', 6'), 8.61 (s, 1H, H-11), 10.00 (s, 1H, NH); LC-MS, *m/z* = 567 [M+1]; Anal. calcd. for C₂₅H₁₇BrFN₅O₃S: C, 53.01; H, 3.03; Br, 14.11; F, 3.35; N, 12.36; S, 5.66; Found: C, 53.04; H, 3.03; Br, 14.11; F, 3.35; N, 12.34; S, 5.65.

N-(3-Fluorophenyl)-2-[(10-bromo-3-(4'-methoxyphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.25). Yield – 81.7%. M.p. – 288-290°C; ¹H NMR δ: 3.91 (s, 3H, -OCH₃), 4.21 (s, 2H, -SCH₂), 6.80 (dd, *J* = 9.4, 7.8 Hz, 1H, 6-Ph H-4'), 7.09 (d, *J* = 7.6 Hz, 2H, 3-Ph H-2', 5'), 7.42-7.23 (m, 2H, 6-Ph H-5', 6'), 7.59 (dd, *J* = 11.3, 0.7 Hz, 1H, 6-Ph H-2'), 7.67 (d, *J* = 7.8 Hz, 1H, H-8), 8.01 (d, *J* = 7.8 Hz, 1H, H-9), 8.43 (d, *J* = 8.0 Hz, 2H, 3-Ph H-2', 6'), 8.53 (s, 1H, H-11), 10.53 (s, 1H, NH); LC-MS, *m/z* = 568 [M+2]; Anal. calcd. for C₂₅H₁₇BrFN₅O₃S: C, 53.01; H, 3.03; Br, 14.11; F, 3.35; N, 12.36; S, 5.66; Found: C, 53.04; H, 3.03; Br, 14.11; F, 3.35; N, 12.34; S, 5.65.

2-[(10-Bromo-3-(4'-fluorophenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.26). Yield – 66.7%. M.p. – 289-291°C; LC-MS, *m/z* = 463 [M+3]; Anal. calcd. for C₁₈H₁₁BrFN₅O₂S: C, 46.97; H, 2.41; N, 15.22; S, 6.97; Found: C, 46.96; H, 2.41; N, 15.22; S, 6.98.

1.4. General procedure for the synthesis of 2-[(8-*R*₁-9-*R*₂-10-*R*₃-3-*R*-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamides (5.1-5.18).

Method A. Add 0.75 g (10 mmol) of chloroacetonitrile to the suspension of the proper potassium 8-*R*₁-9-*R*₂-10-*R*₃-3-*R*-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-thiolates (2.1-2.18) (10 mmol) in 20 ml of propanol-2 and reflux for 1-1.5 hours. Cool the resulted mixture, filter the solid and dry. Crystallize the compounds from DMF-water.

Method B. Stir the mixture of 10 mmol of the proper 2-[(8-*R*₁-9-*R*₂-10-*R*₃-3-*R*-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamides (4.2, 4.5, 4.8, 4.12), 0.35 g (0.6 mmol) NaCl, 25 ml anhydrous dichloroethane, 1.0 g (6.5 mmol) phosphorous oxychloride and 1-2 drops of pyridine when heating (84°C) for 50 min. Then

increase the temperature to 88°C and continue to stir for 4 hours. Evaporate the solvent under vacuum, recrystallize the solid obtained from DMA-water.

2-[(3-Methyl-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetoneitrile (5.1). Yield – 99.3% (Method A). M.p. – 279-281°C; ¹H NMR δ: 2.44 (s, 3H, CH₃), 4.28 (s, 2H, -SCH₂), 7.68 (t, *J* = 7.4 Hz, 1H, H-10), 7.86 (d, *J* = 8.2 Hz, 1H, H-8), 7.97 (t, *J* = 8.2 Hz, 1H, H-9), 8.56 (d, *J* = 7.8 Hz, 1H, H-11); LC-MS, *m/z* = 284 [M+1], 286 [M+3]; Anal. calcd. for C₁₃H₉N₅OS: C, 55.11; H, 3.20; N, 24.72; S, 11.32; Found: C, 55.10; H, 3.20; N, 24.72; S, 11.33.

2-[(3-Phenyl-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetoneitrile (5.2). Yield – 99.6% (Method A); 68.7% (Method B). M.p. – 257-259°C; ¹H NMR δ: 4.33 (s, 2H, -SCH₂), 7.63-7.48 (m, 3H, 3-Ph H-3', 4', 5'), 7.70 (t, *J* = 7.5 Hz, 1H, H-10), 7.87 (d, *J* = 7.8 Hz, 1H, H-8), 7.98 (t, *J* = 7.4 Hz, 1H, H-9), 8.32 (d, *J* = 7.8 Hz, 2H, 3-Ph H-2', 6'), 8.58 (d, *J* = 7.8 Hz, 1H, H-11); LC-MS, *m/z* = 346 [M+1], 348 [M+3]; Anal. calcd. for C₁₈H₁₁N₅OS: C, 62.60; H, 3.21; N, 20.28; S, 9.28; Found: C, 62.62; H, 3.20; N, 20.28; S, 9.27.

2-[(3-(3',4'-Dimethylphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetoneitrile (5.3). Yield – 99.7% (Method A). M.p. – 258-261°C; ¹H NMR δ: 2.35 (s, 3H, 3-Ph 3-CH₃), 2.38 (s, 3H, 3-Ph 4-CH₃), 4.31 (s, 2H, -SCH₂), 7.26 (d, *J* = 7.9 Hz, 1H, 3-Ph H-5'), 7.70 (t, *J* = 7.4 Hz, 1H, H-10), 7.87 (d, *J* = 8.0 Hz, 1H, H-8), 7.97 (t, *J* = 7.4 Hz, 1H, H-9), 8.05 (d, *J* = 7.8 Hz, 1H, 3-Ph H-6'), 8.10 (s, 1H, 3-Ph H-2'), 8.57 (d, *J* = 7.8 Hz, 1H, H-11); LC-MS, *m/z* = 374 [M+1], 376 [M+3]; Anal. calcd. for C₂₀H₁₅N₅OS: C, 64.33; H, 4.05; N, 18.75; S, 8.59; Found: C, 64.34; H, 4.05; N, 18.74; S, 8.59.

2-[(3-(4'-*i*-Propylphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetoneitrile (5.4). Yield – 99.9% (Method A). M.p. – 176-178°C; ¹H NMR δ: 1.32 (d, *J* = 5.5 Hz, 6H, -CH(CH₃)₂), 2.59-2.53 (m, 1H, -CH(CH₃)₂), 4.32 (s, 2H, -SCH₂), 7.37 (d, *J* = 7.8 Hz, 2H, 3-Ph H-3', 5'), 7.69 (t, *J* = 7.3 Hz, 1H, H-10), 7.87 (d, *J* = 7.9 Hz, 1H, H-8), 7.97 (t, *J* = 7.2 Hz, 1H, H-9), 8.25 (d, *J* = 7.7 Hz, 2H, 3-Ph H-2', 6'), 8.57 (d, *J* = 7.7 Hz, 1H, H-11); LC-MS, *m/z* = 388 [M+1], 390 [M+3]; Anal. calcd. for C₂₁H₁₇N₅OS: C, 65.10; H, 4.42; N, 18.08; S, 8.28; Found: C, 65.10; H, 4.41; N, 18.09; S, 8.28.

2-[(3-(4'-(*tert*-Butyl)phenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetoneitrile (5.5). Yield – 96.0% (Method A); 73.7% (Method B). M.p. – 256-258°C; ¹H NMR δ: 1.39 (s, 9H, -C(CH₃)₃), 4.32 (s, 2H, -SCH₂), 7.53 (d, *J* = 8.0 Hz, 2H, 3-Ph H-3', 5'), 7.69 (t, *J* = 7.4 Hz, 1H, H-10), 7.86 (d, *J* = 7.9 Hz, 1H, H-8), 7.97 (t, *J* = 7.4 Hz, 1H, H-9), 8.26 (d, *J* = 8.1 Hz, 2H, 3-Ph H-2', 6'), 8.57 (d, *J* = 7.9 Hz, 1H, H-11); LC-MS, *m/z* = 402 [M+1], 404 [M+3]; Anal. calcd. for C₂₂H₁₉N₅OS: C, 65.81; H, 4.77; N, 17.44; O, 3.99; S, 7.99; Found: C, 65.80; H, 4.77; N, 17.44; O, 3.99; S, 8.00.

2-[(3-(4'-Methoxyphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetoneitrile (5.6). Yield – 82.0% (Method A). M.p. – 272-274°C; ¹H NMR δ: 3.90 (s, 3H, -OCH₃), 4.32 (s, 2H, -SCH₂), 7.05 (d, *J* = 7.3 Hz, 2H, 3-Ph H-3', 5'), 7.70 (t, *J* = 7.6 Hz, 1H, H-10), 7.88 (d, *J* = 7.6 Hz, 1H, H-8), 7.98 (d, *J* = 7.6 Hz, 1H, H-9), 8.39 (d, *J* = 7.6 Hz, 2H, 3-Ph H-2', 6'), 8.58 (d, *J* = 7.4 Hz, 1H, H-11); LC-MS, *m/z* = 376 [M+1], 378 [M+3]; Anal. calcd. for C₁₉H₁₃N₅O₂S: C, 60.79; H, 3.49; N, 18.66; O, 8.52; S, 8.54; Found: C, 60.80; H, 3.49; N, 18.66; O, 8.52; S, 8.53.

2-[(3-(4'-Ethoxyphenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetonitrile (5.7). Yield – 99.9% (Method A). M.p. – 268-270°C; ¹H NMR δ: 1.45 (t, *J* = 5.6 Hz, 3H, -OCH₂CH₃), 4.15 (dd, *J* = 11.1, 5.4 Hz, 2H, -OCH₂CH₃), 4.32 (s, 2H, -SCH₂), 7.02 (d, *J* = 7.6 Hz, 2H, 3-Ph H-3', 5'), 7.71 (t, *J* = 7.3 Hz, 1H, H-10), 7.88 (d, *J* = 7.3 Hz, 1H, H-8), 7.98 (t, *J* = 7.3 Hz, 1H, H-9), 8.38 (d, *J* = 7.7 Hz, 2H, 3-Ph H-2', 6'), 8.58 (d, *J* = 7.3 Hz, 1H, H-11); LC-MS, *m/z* = 390 [M+1], 392 [M+3]; Anal. calcd. for C₂₀H₁₅N₅O₂S: C, 61.68; H, 3.88; N, 17.98; S, 8.23; Found: C, 61.67; H, 3.88; N, 17.98; S, 8.24.

2-[(3-(4'-Fluorophenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetonitrile (5.8). Yield – 99.9% (Method A); 81.3% (Method B). M.p. – 266-268°C; ¹H NMR δ: 4.33 (s, 2H, -SCH₂), 7.29 (t, *J* = 8.5 Hz, 2H, 3-Ph H-3', 5'), 7.71 (t, *J* = 7.4 Hz, 1H, H-10), 7.89 (d, *J* = 8.0 Hz, 1H, H-8), 7.99 (t, *J* = 7.3 Hz, 1H, H-9), 8.43 (dd, *J* = 7.5, 5.9 Hz, 2H, 3-Ph H-2', 6'), 8.59 (d, *J* = 7.9 Hz, 1H, H-11); LC-MS, *m/z* = 364 [M+1], 366 [M+3]; Anal. calcd. for C₁₈H₁₀FN₅O₂S: C, 59.50; H, 2.77; N, 19.27; S, 8.82; Found: C, 59.51; H, 2.77; N, 19.27; S, 8.81.

2-[(8-Methyl-3-phenyl-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetonitrile (5.9). Yield – 98.67% (Method A). M.p. – 255-257°C; ¹H NMR δ: 2.76 (s, 3H, -CH₃), 4.32 (s, 2H, -SCH₂), 7.63-7.46 (m, 4H, H-10, 3-Ph H-3', 4', 5'), 7.81 (d, *J* = 5.6 Hz, 1H, H-9), 8.31 (d, *J* = 8.8 Hz, 2H, 3-Ph H-2', 6'), 8.41 (d, *J* = 7.8 Hz, 1H, H-11); LC-MS, *m/z* = 360 [M+1], 362 [M+3]; Anal. calcd. for C₁₉H₁₃N₅O₂S: C, 63.49; H, 3.65; N, 19.49; S, 8.92; Found: C, 63.48; H, 3.65; N, 19.49; S, 8.93.

2-[(9-Fluoro-3-phenyl-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetonitrile (5.10). Yield – 88.0% (Method A); 71.9% (Method B). M.p. – 250-253°C; ¹H NMR δ: 4.33 (s, 2H, -SCH₂), 7.63-7.45 (m, *J* = 14.9, 7.7 Hz, 5H, H-8, 10, 3-Ph H-3', 4', 5'), 8.29 (d, *J* = 7.2 Hz, 2H, 3-Ph H-2', 6'), 8.64 (dd, *J* = 8.7, 5.9 Hz, 1H, H-11); LC-MS, *m/z* = 364 [M+1], 366 [M+3]; Anal. calcd. for C₁₈H₁₀FN₅O₂S: C, 59.50; H, 2.77; N, 19.27; S, 8.82; Found: C, 59.51; H, 2.77; N, 19.26; S, 8.82.

2-[(9-Fluoro-3-(4'-methoxyphenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetonitrile (5.11). Yield – 74.67% (Method A). M.p. – 273-275°C; ¹H NMR δ: 3.90 (s, 3H, -CH₃), 4.32 (s, 2H, -SCH₂), 7.04 (d, *J* = 8.2 Hz, 2H, 3-Ph H-3', 5'), 7.50 (dd, *J* = 8.6, 6.6 Hz, 1H, H-10), 7.57 (d, *J* = 7.6 Hz, 1H, H-8), 8.36 (d, *J* = 8.3 Hz, 2H, 3-Ph H-2', 6'), 8.63 (dd, *J* = 8.7, 5.9 Hz, 1H, H-11); LC-MS, *m/z* = 394 [M+1], 396 [M+3]; Anal. calcd. for C₁₉H₁₂FN₅O₂S: C, 58.01; H, 3.07; N, 17.80; S, 8.15; Found: C, 58.02; H, 3.07; N, 17.80; S, 8.14.

2-[(9-Fluoro-3-(4'-fluorophenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetonitrile (5.12). Yield – 48.0% (Method A). M.p. – 258-260°C; ¹H NMR δ: 4.33 (s, 2H, -SCH₂), 7.29 (t, *J* = 8.4 Hz, 2H, 3-Ph H-3', 5'), 7.51 (t, *J* = 7.5 Hz, 1H, H-10), 7.58 (d, *J* = 9.2 Hz, 1H, H-8), 8.40 (t, *J* = 5.7 Hz, 2H, 3-Ph H-2', 6'), 8.63 (dd, *J* = 8.3, 6.0 Hz, 1H, H-11); LC-MS, *m/z* = 382 [M+1], 384 [M+3]; Anal. calcd. for C₁₈H₉F₂N₅O₂S: C, 56.69; H, 2.38; N, 18.36; S, 8.41; Found: C, 56.68; H, 2.38; N, 18.37; S, 8.41.

2-[(10-Chloro-3-phenyl-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetonitrile (5.13). Yield – 69.33% (Method A). M.p. – 262-264°C; ¹H NMR δ: 4.33 (s, 2H, -SCH₂), 7.65-7.37 (m, 3H, 3-Ph H-3', 4', 5'), 7.88 (d, *J* = 8.6 Hz, 1H, H-8), 7.95 (d, *J* = 8.6 Hz, 1H, H-9), 8.31 (d, *J* = 7.3 Hz, 2H, 3-Ph H-2', 6'), 8.50 (s, 1H, H-11); LC-MS, *m/z* = 380 [M+1], 382 [M+3], 383 [M+4]; Anal. calcd. for

C₁₈H₁₀ClN₅O₂S: C, 56.92; H, 2.65; N, 18.44; S, 8.44; Found: C, 56.92; H, 2.65; N, 18.43; S, 8.45.

2-[(10-Chloro-3-(4'-methylphenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetonitrile (5.14). Yield – 64.0% (Method A). M.p. – 250-253°C; ¹H NMR δ: 2.46 (s, 3H, -CH₃), 4.32 (s, 2H, -SCH₂), 7.33 (d, *J* = 7.8 Hz, 2H, 3-Ph H-3', 5'), 7.87 (d, *J* = 8.6 Hz, 1H, H-8), 7.93 (d, *J* = 8.6 Hz, 1H, H-9), 8.24 (d, *J* = 7.8 Hz, 2H, 3-Ph H-2', 6'), 8.49 (s, 1H, H-11); Anal. calcd. for C₁₉H₁₂ClN₅O₂S: C, 57.94; H, 3.07; N, 17.78; S, 8.14; Found: C, 57.93; H, 3.07; N, 17.79; S, 8.14.

2-[(10-Chloro-3-(4'-fluorophenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetonitrile (5.15). Yield – 69.33% (Method A). M.p. – 270-272°C; ¹H NMR δ: 4.33 (s, 2H, -SCH₂), 7.29 (t, *J* = 8.6 Hz, 2H, 3-Ph H-3', 5'), 7.88 (d, *J* = 8.7 Hz, 1H, H-8), 7.95 (d, *J* = 8.5 Hz, 1H, H-9), 8.42 (dd, *J* = 8.0, 5.8 Hz, 2H, 3-Ph H-2', 6'), 8.49 (s, 1H, H-11); LC-MS, *m/z* = 398 [M+1], 400 [M+3], 401 [M+4]; Anal. calcd. for C₁₈H₉ClFN₅O₂S: C, 54.35; H, 2.28; N, 17.60; S, 8.06; Found: C, 54.36; H, 2.27; N, 17.60; S, 8.06.

2-[(10-Bromo-3-phenyl-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetonitrile (5.16). Yield – 96.7% (Method A). M.p. – 260-252°C; ¹H NMR δ: 4.33 (s, 2H, -SCH₂), 7.66-7.41 (m, 3H, 3-Ph H-3', 4', 5'), 7.81 (d, *J* = 8.6 Hz, 1H, H-8), 8.08 (d, *J* = 8.6 Hz, 1H, H-9), 8.31 (d, *J* = 7.3 Hz, 2H, 3-Ph H-2', 6'), 8.65 (s, 1H, H-11); LC-MS, *m/z* = 425 [M+1]; Anal. calcd. for C₁₈H₁₀BrN₅O₂S: C, 50.96; H, 2.38; N, 16.51; S, 7.56; Found: C, 50.97; H, 2.38; N, 16.50; S, 7.56.

2-[(10-Bromo-3-(4'-methylphenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetonitrile (5.17). Yield – 77.2% (Method A). M.p. – 268-270°C; ¹H NMR δ: 2.46 (s, 3H, -CH₃), 4.32 (s, 2H, -SCH₂), 6.32 (d, *J* = 7.9 Hz, 2H, 3-Ph H-3', 5'), 7.80 (d, *J* = 8.6 Hz, 1H, H-8), 8.06 (dd, *J* = 8.6, 1.6 Hz, 1H, H-9), 8.24 (d, *J* = 8.0 Hz, 2H, 3-Ph H-2', 6'), 8.63 (s, 1H, H-11); LC-MS, *m/z* = 439 [M+1]; Anal. calcd. for C₁₉H₁₂BrN₅O₂S: C, 52.07; H, 2.76; N, 15.98; S, 7.32; Found: C, 52.06; H, 2.76; N, 15.98; S, 7.33.

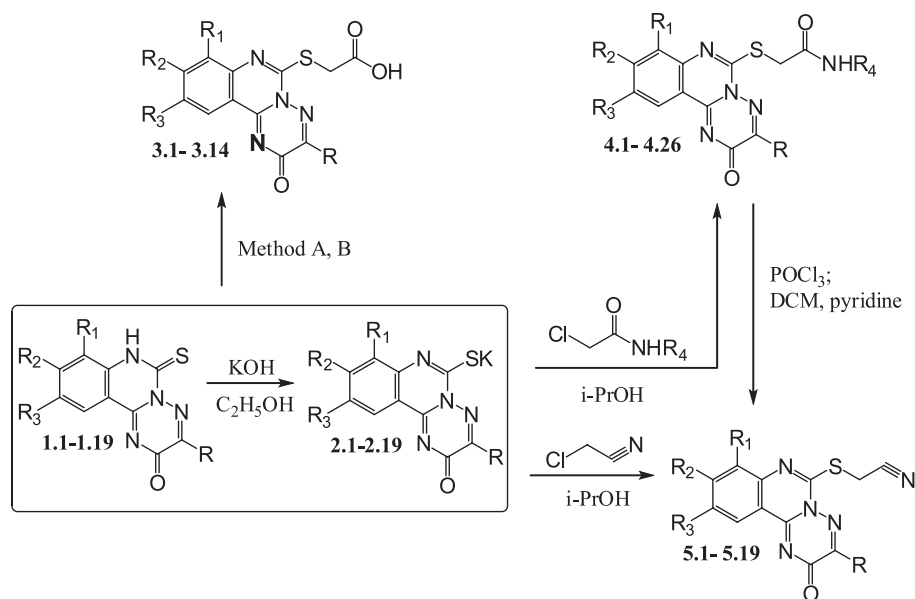
2-[(10-Bromo-3-(4'-methoxyphenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetonitrile (5.18). Yield – 88.0% (Method A). M.p. – 266-268°C; ¹H NMR δ: 3.88 (s, 3H, -CH₃), 4.30 (s, 2H, -SCH₂), 7.03 (d, 2H, 3-Ph H-3', 5'), 7.81 (d, 1H, H-8), 8.04 (d, 1H, H-9), 8.37 (d, 2H, 3-Ph H-2', 6'), 8.62 (s, 1H, H-11); LC-MS, *m/z* = 455 [M+1], 456 [M+2], 458 [M+4]; Anal. calcd. for C₁₉H₁₂BrN₅O₂S: C, 50.23; H, 2.66; N, 15.42; S, 7.06; Found: C, 50.22; H, 2.66; N, 15.43; S, 7.06.

2-[(10-Bromo-3-(4'-fluorophenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetonitrile (5.19). Yield – 64.0%. M.p. – 259-261°C; ¹H NMR δ: 4.33 (s, 2H, -SCH₂), 7.29 (t, *J* = 8.4 Hz, 3H, 3-Ph H-3', 5'), 7.81 (d, *J* = 8.6 Hz, 1H, H-8), 8.07 (d, *J* = 8.3 Hz, 1H, H-9), 8.42 (dd, *J* = 7.3, 5.8 Hz, 2H, 3-Ph H-2', 6'), 8.64 (s, 1H, H-11); LC-MS, *m/z* = 446 [M+4]; Anal. calcd. for C₁₈H₉BrFN₅O₂S: C, 48.88; H, 2.05; N, 15.84; S, 7.25; Found: C, 48.89; H, 2.05; N, 15.84; S, 7.24.

2. Pharmacology

Antimicrobial and antifungal test

Sensitivity of microorganisms to the compounds synthesized was assessed according to the methods described [9]. The assay was conducted on the Mueller-Hinton medium by two-fold serial dilution of compounds in 1 ml. After that 0.1 ml of microbial seeding (10⁶ cells/ml) was added. The minimal inhibitory concentration of com-



Method A: ClCH₂COOH, NaOH, H₂O; Method B: ClCH₂COOH, Na, C₂H₅OH; R=C₂H₅OH; R=CH₃, Ph, 4-CH₃Ph, 3,4-(CH₃)₂Ph, 4-C₂H₅Ph, 4-(CH₃)₂CHPh, 4-(CH₃)₃CPh, 4-CH₃OPh, 4-C₂H₅OPh, 4-FPh; R₁=H, CH₃, Br; R₂=H, F, Br; R₃=H, Cl, Br; R₄=H, 2-FPh, 3-FPh, 4-BrPh, 4-CH₃OPh, 4-CF₃Ph

Scheme. Synthesis of [(8-R₁-9-R₂-10-R₃-3-R-2-oxo-2H-[1,2,4]triazino[2,3-c]-quinazolin-6-yl)thio]acetic acids and the functional derivatives.

pounds was determined by the absence of visual growth in the test tube with the minimal concentration of the substance, the minimal bactericidal/fungicidal concentration was determined by the absence of growth on agar after inoculation of the microorganism from transparent test-tubes. Dimethylsulfoxide was used as a solvent, the initial solution concentration was 1 mg/ml. For preliminary screening the standard test cultures such as *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 885-653 were used. All test strains were received from the bacteriological laboratory at the Zaporizhzhya Regional Laboratory Centre of the State Sanitary and Epidemiological Service of Ukraine. Nitrofurazone, trimetoprim and ketoconazole were used as reference compounds with the proved antibacterial/antifungal activity. Additional quality control of the culture medium and solvents was conducted by the methods commonly used.

Results and Discussion

1. Chemistry

As initial compounds, 3-R-6-thio-6,7-dihydro-2H-[1,2,4]triazino[2,3-c]-quinazolin-2-ones (**1**) and their potassium salts were used; the salts were obtained according to the known protocols of 6-R-3-(3-R₁-4-R₂-5-R₃-2-aminophenyl)-1,2,4-triazin-5(2H)-ones with carbon disulfide, potassium hydroxide in the ethyl alcohol medium and potassium ethylxanthate in propanol-2 [7, 8].

The synthesis of [(8-R₁-9-R₂-10-R₃-3-R-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetic acids (**3**) was conducted via alkylation of potassium thiolates with chloroacetic acids in propanol-2 or propanol-2 – water in the presence of the equivalent amount of sodium hydroxide (Scheme). After acidification of the reaction mixture (pH 3-4), the solids of the corresponding acids were

formed with high yields. Amides **4** were synthesized by alkylation of potassium thiolates **2** with chloroacetamide or N-R₄-chloroacetamides in propanol-2. Unfortunately, we failed to obtain amides **4** by amination of the corresponding ester because of low reactivity of the compounds mentioned.

Synthesis of nitriles **5** by dehydration of the proper amides **4** with phosphorous-oxychloride in dichloromethane was not successful in all cases. The fact mentioned was caused by low yields and problems with isolation of the target compound from the reaction mixture. Thus, nitriles **5** were synthesized by interaction of potassium thiolates with chloroacetonitrile in propanol-2.

We noted that thionamides **1** were also alkylated by chloroacetic acid and their derivatives in propanol-2 in the presence of sodium hydroxide. The reaction easily flows for 1-1.5 h., elongation of the process did not lead to the increase of the yields.

The purity of the compounds synthesized was confirmed by LC-MS, the structure by elemental analysis, ¹H and ¹³C NMR-spectrometry.

In LC-MS spectra of compounds **3**, **4** and **5** in most cases the positive ions [M+1] and [M+3] were observed, the m/z values of the ions mentioned corresponded to the molecular weight of the target compounds. In LC-MS spectra acids **3.2-3.4** we have found the signals, which according to their m/z value belong to the fragmentary ion [M-CH₂COOH]⁺, and it additionally confirms the structure of the acids synthesized.

In ¹H NMR-spectra of compounds **3**, **4** and the singlet signal of the SCH₂-group at 4.33-3.89 ppm, the chemical shift of the signal was caused by the nature of the substituent at the carbonyl group. The signal of the proton mentioned was observed in the lower field of ¹H-NMR of the corresponding nitriles **5** (4.33-4.32 ppm). The pro-

Table

The antimicrobial and antifungal activity of the compounds synthesized

Comp. No.	R*	R ₁	R ₂	R ₃	E. coli		S. aureus		P. aeruginosa		C. albicans	
					MIC, µg/ml	MBC, µg/ml	MIC, µg/ml	MBC, µg/ml	MIC, µg/ml	MBC, µg/ml	MIC, µg/ml	MFC, µg/ml
3.3	4-(Me)C ₆ H ₄	H	H	H	100	100	25	100	50	100	50	50
3.4	3,4-(Me) ₂ C ₆ H ₃	H	H	H	100	200	12.5	100	50	100	50	100
3.5	4-(Et)C ₆ H ₄	H	H	H	100	200	12.5	50	100	200	50	100
3.6	4-(i-Pr)-C ₆ H ₄	H	H	H	100	200	12.5	25	100	100	50	100
3.14	4-F-C ₆ H ₄	H	H	Br	200	200	12.5	50	100	200	100	100
4.1	Me	H	H	H	50	100	100	200	200	200	25	100
4.5	4-(Et)C ₆ H ₄	H	H	H	100	100	25	100	100	200	50	100
4.6	4-(tert-Bu)C ₆ H ₄	H	H	H	100	200	25	100	100	200	50	100
4.7	4-(MeO)C ₆ H ₄	H	H	H	100	200	25	25	100	200	25	50
4.8	4-(EtO)C ₆ H ₅	H	H	H	100	100	12.5	50	100	200	50	100
4.11	Ph	Me	H	H	100	100	50	100	50	100	25	100
4.12	4-F-C ₆ H ₄	Br	H	H	100	100	50	200	100	200	25	50
4.13	Ph	H	F	H	100	200	12.5	100	100	200	12.5	12.5
4.14	4-(MeO)C ₆ H ₄	H	F	H	200	200	12.5	25	200	200	12.5	25
4.16	4-FC ₆ H ₅	H	F	H	100	200	25	50	200	200	25	50
4.26	4-F-C ₆ H ₄	H	H	Br	50	100	100	200	200	200	25	100
5.10	Ph	H	F	H	100	200	50	100	100	200	12.5	25
5.11	4-(MeO)C ₆ H ₄	H	F	H	100	200	50	100	100	200	25	50
5.13	Ph	H	H	Cl	25	100	100	200	50	100	50	100
5.14	4-MeC ₆ H ₄	H	H	Cl	25	100	100	200	50	100	50	100
Nitrofurantoin					1.5	–	6.25	–	6.25	–	25.0	–
Trimethoprim					50	50	31.2	62.5	62.5	125	62.5	125
Ketoconazole					–	–	–	–	–	–	25	–

* – compounds **3.1, 3.2, 3.7-3.13, 4.2-4.4, 4.9, 4.10, 4.15, 4.17-4.25, 5.1-5.9, 5.15-5.19** exhibit the antibacterial activity ≤50 µg/ml.

ton of the carboxylic group of acids was observed at 13.92-12.90 ppm. Protons of the primary amide group of compounds **4** as result of the C=O group effect were observed as two non-equivalent one-proton singlets at 7.30-7.08 ppm and 7.58-7.53 ppm. For the secondary amides **4** the amide protons were observed as a singlet at 10.59-10.00 ppm (for **4.9, 4.16, 4.24, 4.25**) or 8.82-8.5 ppm (**4.19, 4.21, 4.22**). Compounds (**4.19, 4.21, 4.22**) were also characterized by a two-proton doublet of the NCH₃ fragment in ¹H NMR spectra. Aromatic protons of the non-substituted triazinoquinazoline cycle of compounds **3, 4, 5** formed systems with two one-proton doublets H-8 and H-11 and two one-proton triplets H-9 and H-10. Signals of aromatic protons of the *para*-substituted phenyl moiety in position 3 of compounds **3, 4** and **5** were observed as the A₂B₂-system consisting of two-proton doublets (H-3, H-5 and H-2, H-6), at the same time, signals of the unsubstituted one were observed as a multiplet (H-3, H-4, H-5) and a two-proton doublet.

Signals of *sp*³-hybrid carbons belonging to CH₃ (18.19 ppm), SCH₂ (34.21-38.94 ppm), CH₃O (55.94 ppm) were observed in the high field of ¹³C NMR-spectra of some compounds. According to ¹³C NMR spectra the most deshielded carbons were located in the -COOH-group (170.04-170.08 ppm) and positions 2 and 6 of [1,2,4]triazino[2,3-*c*]quinazoline system.

2. Antimicrobial and antifungal activities

The antimicrobial assay has shown that the compounds synthesized exhibit the antibacterial and antifungal activity against the strains studied. Thus, 2-[(3-methyl-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetic acids (**3.1**, MIC 50-200 µg/ml) exhibit a moderate action against *E. coli*, *S. aureus* and *P. aeruginosa*, but a high activity against *C. albicans* (MIC 50 µg/ml, Table). Chemical modification of compound **3.1** via changing the methyl group in position 3 into the phenyl moiety (**3.2**) did not lead to the activity increase. Introduction of fluorine (**3.9**) and alkoxy groups (**3.7, 3.8**) in the phenyl moiety also did not lead to the increase of the antibacterial and antifungal activity. At the same time 4-alkylphenyl derivatives (**3.3-3.6**) exhibit a high inhibiting effect against *S. aureus* (MIC 12.5-25 µg/ml). We noticed that the increase in activity was observed in case of introduction of the additional methyl group (compound **3.4**), elongation (**3.5**) and branching (**3.6**) of the alkyl group in the phenyl substituent (Table). Introduction of the addition substituent (fluoro-, chloro-, bromo- and methyl group) in position 8, 9, 10 did not cause essential changes of the antimicrobial and antifungal activity. Only compound **3.14** containing bromine in position 10 and (4-fluoro)phenyl in position 3 exhibits a high activity against *S. aureus* (MIC 12.5 µg/ml).

Following modification of the carboxylic group (compounds **3.1-3.14**) in the amide fragment (**4.1-4.26**) resulted in increasing the antibacterial and antifungal activity against the strains studied. We noticed that some amides (**4.1, 4.3, 4.4, 4.9, 4.15, 4.17, 4.18, 4.20, 4.21, 4.26**) unlike acids (**3.1-3.15**) exhibited the antibacterial activity against *E. coli* (MIC 50 µg/ml) at the same level as trimetoprim (MIC 50 µg/ml). Moreover, amides **4.1-4.26** showed a high antifungal action against *C. albicans* (MIC 12.5-50 µg/ml) exceeding the activity of trimetoprim (MIC 62.5 µg/ml) and was comparable to nitrofurantoin (MIC 25.0 µg/ml, compounds **4.1, 4.7, 4.11, 4.14, 4.16, 4.26**). A considerable attention as to antifungal agents should be paid to compounds **4.13** and **4.14** (MIC 12.5 µg/ml) exceeding the activity of ketoconazole (MIC 25 µg/ml).

We noted that as in case of acids amides **4.1-4.26** were more active against *S. aureus* (MIC 12.5-100 µg/ml). The modification of compound **4.1** was conducted via changing the methyl group in position 3 of the triazinoquinazoline system into phenyl (**4.2**), 4-alkylphenyl (**4.3, 4.5-4.6**), 3,4-dimethylphenyl (**4.4**) and 4-alkoxyphenyl (**4.7, 4.8**) results in the activity increase. Unlike acids (**3.11, 3.12**), amides (**4.13-4.16**) containing a fluorine atom in position 9 were more active against *S. aureus*. Substitution of fluorine (**4.13-4.16**) into bromine (**4.17**) caused insignificant decrease in activity (MIC 50 µg/ml). The complete loss of activity was observed in case of translocation of bromine from 9 to 10 position (compounds **4.22, 4.23, 4.26**; MIC 50-100 µg/ml) and introduction of chlorine (**4.19** MIC 50 µg/ml). Introduction of the N-substituted amide group (compounds **4.9, 4.15, 4.18, 4.20, 4.21, 4.25, 4.26**) irrespective of the substituent and its location in the molecule did not affect or cause insignificant decrease of action against *S. aureus*.

Nitriles (**5.1-5.19**) unlike acids (**3.1-3.15**) and amides (**4.1-4.26**) were active against *P. aeruginosa* (MIC

50-100 µg/ml). Among all the compounds studied only compounds **5.13** and **5.14** revealed a high antimicrobial action against *E. coli* (MIC 25 µg/ml). At the same time the inhibitory activity of nitriles (**5.1-5.19**) against *S. aureus* comparing to acids and amides were insignificant (MIC 50-200 µg/ml). Nitriles (**5.1-5.19**) also exhibited a high inhibitory action against *C. albicans* (MIC 12.5-100 µg/ml). Among the compounds studied our attention was focused on compound **5.10** (MIC 12.5 µg/ml) exceeding ketoconazole (MIC 25 µg/ml) in its activity.

Thus, the antimicrobial and antifungal activity of 2-[8-R₁-9-R₂-10-R₃-3-R-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazoline-6-yl)thio]acetic acids studied and their derivatives was caused by the nature of substituents in position 6, substituents of the phenyl moiety in position and substituents in positions 8, 9, 10 of the triazinoquinazoline system. We have found that the presence of 4-alkylphenyl (compounds **3.3-3.6, 4.3-4.6**; MIC 12.5-25 µg/ml), 4-alkoxyphenyl (**4.7, 4.8**; MIC 12.5-50 µg/ml) substituents in position 3 and fluorine in position 9 **4.13-4.16**; MIC 12.5-25 µg/ml) contributes positively to the high activity against *S. aureus*, the presence of fluorine in position 9 also promotes the activity against *C. albicans* (**4.13, 4.14**; MIC 12.5 µg/ml).

CONCLUSIONS

In the present paper 50 new derivatives of 2-[8-R₁-9-R₂-10-R₃-3-R-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazoline-6-yl)thio]acetic acids have been described. The compounds synthesized have been tested for the antimicrobial and antifungal activity using standard test cultures: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 885-653. It has been shown that the compounds synthesized exhibit a high antimicrobial activity against *St. aureus* (compounds **3.3-3.6, 4.3-4.6, 4.7, 4.8, 4.13-4.16**; MIC 12.5-25 µg/ml) and *C. albicans* (compounds **4.13, 4.14**; MIC 12.5 µg/ml). The "structure-activity" relationship has been discussed.

REFERENCES

1. Antipenko L.N., Karpenko A.V., Kovalenko S.I. et al. // Arch. Pharm. – 2009. – Vol. 342, Iss. 11. – P. 651-662.
2. Antipenko L., Karpenko A., Kovalenko S. et al. // Chem. Pharm. Bull. (Tokyo). – 2009. – Vol. 57, №6. – P. 580-585.
3. Available from: <http://www.drugbank.ca/structures/search/>.
4. Baruah B., Dasu K., Vaitilingam B. et al. // Bioorg. Med. Chem. – 2004. – Vol. 12, Iss. 9. – P. 1991-1994.
5. Bedi P.M.S., Kumar V., Mahajan M.P. // Bioorg. Med. Chem. Lett. – 2004. – Vol. 14, Iss. 20. – P. 5211-5213.
6. Berest G.G., Kovalenko S.I., Nosulenko I.S. et al. // Sci. Pharm. – 2012. – Vol. 80, Iss. 1. – P. 37-65.
7. Berest G.G., Kovalenko S.I., Sinyak R.S. et al. // J. of Org. and Pharmac. Chem. – 2010. – №8. – P. 42-52.
8. Berest G.G., Voskoboynik O.Y., Kovalenko S.I. et al. // Eur. J. Med. Chem. – 2011. – Vol. 46, Iss. 12. – P. 6066-6074.
9. Institute CaLS. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard.
10. Jantová S., Ovádek R., Letašiová S. et al. // Folia Microbiol. (Praha). – 2005. – Vol. 50, Iss. 2. – P. 90-94.
11. Kovalenko S., Nosulenko I., Voskoboynik A. et al. // Med. Chem. Res. – 2013. – Vol. 22, Iss. 6. – P. 2610-2632.
12. Kovalenko S.I., Voskoboynik A.Yu., Berest G.G. et al. // Sci. Pharm. – 2012. – Vol. 80, Iss. 4. – P. 837-865.
13. Kuarm B.S., Reddy Y.T., Madhav J.V. et al. // Bioorg. Med. Chem. Lett. – 2011. – Vol. 21, Iss. 1. – P. 524-527.
14. Lamazzi C., Léonce S., Pfeiffer B. et al. // Bioorg. Med. Chem. Lett. – 2000. – Vol. 10, Iss. 19. – P. 2183-2185.
15. Nasr M.N., Gineinah M.M., El-Bendary E.R. // Arch. Pharm. – 2003. – Vol. 336, Iss. 12. – P. 560-566.

16. Ovadekova R.J.S., Theiszova M., Labuda I. // *J. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech. Repub.* – 2005. – Vol. 149, Iss. 2. – P. 455-459.
17. Rohini R., Muralidhar Reddy P., Shanker K. et al. // *Eur. J. Med. Chem.* – 2010. – Vol. 45, Iss. 3. – P. 1200-1205.
18. Rohini R., Shanker K., Reddy P.M. et al. // *Eur. J. Med. Chem.* – 2009. – Vol. 44, Iss. 8. – P. 3330-3339.

СИНТЕЗ ТА МОДИФІКАЦІЯ 2-[(8-R₁-9-R₂-10-R₃-3-R-2-ОКСО-2Н-[1,2,4]ТРИАЗИНО[2,3-с]ХІНАЗОЛІН-6-ІЛ)ТІО]ОЦТОВИХ КИСЛОТ, СПРЯМОВАНІ НА ПОШУК СПОЛУК З АНТИБАКТЕРІАЛЬНОЮ ТА ПРОТИГРИБКОВОЮ ДІЄЮ

І.С.Носуленко, О.Ю.Воскобойнік, Г.Г.Берест, С.І.Коваленко, О.М.Камишний, Н.М.Поліщук

Ключові слова: синтез; хіназоліни; триазины; антибактеріальна активність; протигрибкова активність

В представленій роботі описано синтез 50 нових похідних 2-[8-R₁-9-R₂-10-R₃-3-R-2-оксо-2Н-[1,2,4]триазино[2,3-с]хіназолін-6-іл)тіо]оцтових кислот. Показано, що алкілювання калій 8-R₁-9-R₂-10-R₃-3-R-2-оксо-2Н-[1,2,4]триазино[2,3-с]хіназолін-6-тіолатів хлороцтовою кислотою, хлорацетамідом, N-R₄-хлорацетамідами та хлорацетонітрилом веде до утворення відповідних 2-[8-R₁-9-R₂-10-R₃-3-R-2-оксо-2Н-[1,2,4]триазино[2,3-с]хіназолін-6-іл)тіо]оцтових кислот, їх амідів та нітрилів. Для відповідних кислот та нітрилів були опрацьовані альтернативні синтетичні підходи. Також були обговорені обмеження у синтезі цільових сполук. Так, було показано, що амід 2-[8-R₁-9-R₂-10-R₃-3-R-2-оксо-2Н-[1,2,4]триазино[2,3-с]хіназолін-6-іл)тіо]оцтових кислот не можуть бути одержані амонізом естерів внаслідок низької реакційної здатності останніх. Заявлено, що синтез нітрилів дегідратацією відповідних амідів хлорокисом фосфору у дихлорометані був успішним не в усіх випадках. Зазначений факт обумовлено вкрай низькими виходами цільових сполук, що пов'язано зі значними проблемами при їх виділенні з реакційної суміші. Структуру синтезованих сполук було визначено за допомогою комплексу сучасних фізико-хімічних методів (¹H, ¹³C NMR, LC-MS-спектрами). Синтезовані сполуки були випробувані на антимікробну та протигрибкову дію з використанням стандартних тест-культур: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 та *Candida albicans* ATCC 885-653. Показано, що сполуки 3.3-3.6, 4.3-4.6, 4.7, 4.8, 4.13-4.16 проявляють виражену активність по відношенню до *St. aureus* (MIC 12,5-25 µg/ml), а сполуки 4.13, 4.14 також по відношенню до *C. albicans* (MIC 12,5 µg/ml). В рамках статті обговорено взаємозв'язок «структура-біологічна дія».

СИНТЕЗ И МОДИФИКАЦИЯ 2-[(8-R₁-9-R₂-10-R₃-3-R-2-ОКСО-2Н-[1,2,4]ТРИАЗИНО[2,3-с]ХИНАЗОЛИН-6-ИЛ)ТИО]УКСУСНЫХ КИСЛОТ, НАПРАВЛЕННЫЕ НА ПОИСК СОЕДИНЕНИЙ С АНТИБАКТЕРИАЛЬНЫМ И ПРОТИВОГРИБКОВЫМ ДЕЙСТВИЕМ

И.С.Носуленко, А.Ю.Воскобойник, Г.Г.Берест, С.И.Коваленко, А.М.Камышний, Н.М.Поліщук

Ключевые слова: синтез; хиназолины; триазины; антибактериальная активность; противогрибковая активность

В представленной работе описан синтез 50 новых производных 2-[8-R₁-9-R₂-10-R₃-3-R-2-оксо-2Н-[1,2,4]триазино[2,3-с]хиназолин-6-ил)тио]уксусных кислот. Показано, что алкилирование калій 8-R₁-9-R₂-10-R₃-3-R-2-оксо-2Н-[1,2,4]триазино[2,3-с]хиназолин-6-тиолатов хлоруксусной кислотой, хлорацетамидом, N-R₄-хлорацетамідами и хлорацетонитрилом приводит к образованию соответствующих 2-[8-R₁-9-R₂-10-R₃-3-R-2-оксо-2Н-[1,2,4]триазино[2,3-с]хиназолин-6-ил)тио]уксусных кислот, их амидов и нитрилов. Для соответствующих кислот и нитрилов были разработаны альтернативные синтетические подходы. Также были обговорены ограничения в синтезе целевых соединений. Так было показано, что амиды 2-[8-R₁-9-R₂-10-R₃-3-R-2-оксо-2Н-[1,2,4]триазино[2,3-с]хиназолин-6-ил)тио]уксусных кислот не могут быть получены амонізом соответствующих эфиров ввиду низкой реакционной активности последних. Заявлено, что синтез нитрилов дегидратацией соответствующих амидов хлоркисью фосфора в дихлорметане был успешен не во всех случаях. Данный факт был обусловлен крайне низкими выходами целевых соединений вследствие значительных проблем при их выделении из реакционных смесей. Структура синтезированных соединений подтверждена с помощью современных физико-химических методов (¹H, ¹³C NMR, LC-MS-спектры). Синтезированные соединения были исследованы на противомикробную и противогрибковую активность с использованием стандартных тест культур: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 и *Candida albicans* ATCC 885-653. Показано, что соединения 3.3-3.6, 4.3-4.6, 4.7, 4.8, 4.13-4.16 проявляют выраженную активность по отношению к *St. aureus* (MIC 12,5-25 µg/ml), а соединения 4.13, 4.14 также по отношению к *C. albicans* (MIC 12,5 µg/ml). Также в рамках статьи обсуждена взаимосвязь «структура-биологическое действие».

Acknowledgements

The authors are grateful to "Enamine Ltd." (Kyiv, Ukraine) for financial support of this work.

Recommended by Doctor of Chemistry, professor M.Ye.Blazheyevskiy

UDC 547.732: 543.242.3: 543.42.062: 543.257

VOLTAMMETRIC DETERMINATION OF CEFOTAXIME USING POTASSIUM PEROXOMONOSULFATE

Yu.Yu.Labuzova

National University of Pharmacy

Key words: voltammetry; cefotaxime; potassium peroxomonosulfate

The presented article is devoted to development of a new procedure for quantitative voltammetric determination of Cefotaxime powder for preparing the solution for injection in the form of the corresponding S-oxide in a weak acidic medium using potassium hydrogenperoxomonosulfate (KHSO_5) as an analytical reagent. Voltammograms of Cefotaxime S-oxide solutions for different concentrations of cephalosporins have been scanned. There are two peaks on the voltammetric curve of the Cefotaxime S-oxide solution: at -0.65 V (that corresponds to potassium peroxomonosulfate) and -1.3 V (the peak height was rising proportionally to Cefotaxime concentrations increase) that has been chosen as analytical. The calibration curve method can be easily applied. Linearity has been studied over the drug concentration range from $1 \cdot 10^{-4}$ to $1 \cdot 10^{-3}\text{ mol L}^{-1}$. The correlation coefficient is $r = 0.999$. Precision and accuracy have been studied by analyzing five replicates of the sample solutions at three concentrations levels. The relative standard deviations calculated were below 1.75% , $\delta \leq 1.1\%$ indicating the excellent precision of the procedure proposed. The Limit of Detection (LOD) and the Limit of Quantification (LOQ) were calculated ($\text{LOD} = 1.2 \cdot 10^{-5}\text{ mol L}^{-1}$ and $\text{LOQ} = 4 \cdot 10^{-5}\text{ mol L}^{-1}$). The voltammetric method proposed is sensitive enough, accurate, precise, replicable and linear to enable determination of lower amounts of a drug. These advantages encourage the application of the method in the routine quality control of Cefotaxime in control and research laboratories.

Cefotaxime (CFTM), which structure is presented in Scheme, is the second generation cephalosporin derivative widely used in clinical therapy of severe infections. Chemically, it is (6R,7R,Z)-3-(Acetoxymethyl)-7-(2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylic acid [5].

Extensive literature survey reveals that a lot of analytical methods are reported for analysis of Cefotaxime like HPLC [13, 14], Capillary electrophoresis [6], Spectrophotometry [1, 11, 12] and Spectrofluorimetry [10].

Polarography, especially differential pulse polarography, and other voltammetric techniques become increasingly important when determining compounds of biological and pharmaceutical significance [8, 9].

Literature dealing with the electroactivity of cephalosporins and resulting analytical applications can be divided into two parts: papers concerning the direct polarographic activity of cephalosporins and papers dealing with polarography of their degradation products after intensive acidic, or alkaline hydrolysis [2-4, 7]. The main electrode reaction responsible for the direct polarographic activity of cephalosporins has been shown to be reduction of the Δ^3 double bond of the cephem nucleus, which is dependent on the presence and nature of the substituent at position 3 [15]. So, Cefotaxime belongs to cephalosporins with a reducible group in the side-chain on C-7 and R^1 in $3\text{-CH}_2\text{R}^1$. But the indirect method of determination of cephalosporins as the corresponding derivatives requires special conditions (heating, intensive alkali or acidic medium) and are long-lasting. It is supposed that the method of cephalosporin deriva-

tives produced by S-oxidation reactions determination is more informative. So, voltammetric determination of Cefotaxime by the reaction of S-oxidation by means of potassium peroxomonosulfate in a weak acidic medium was optimized and proposed for the first time.

Materials and Methods

Reagents and Chemicals

All materials were of the analytical reagent grade, and the solutions were prepared with double-distilled water. Cefotaxime, powder for injection, 1.000 g, was produced by "Pharmaceutical company "Zdorovie" (Kharkiv, Ukraine) Ltd, batch No. 41008.

Peroxomonosulfate (Sigma-Aldrich) was employed as received. The solution of peroxomonosulfate was prepared by dissolving its potassium salt ($\text{K}_2\text{SO}_5 \cdot \text{KHSO}_5 \cdot \text{K}_2\text{SO}_4$) in double-distilled water.

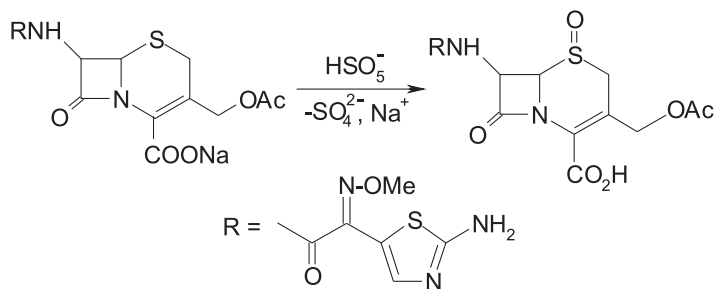
Equipment

Electrochemical behaviour of cefotaxime S-oxide by differential voltammetry using carbosital electrode (CE) (Russia) as an indicating (working) electrode was studied.

Electrochemical measurements were carried out in an ABC-1.1 analyzer (Volta, St. Petersburg) with a three-electrode scheme by the alternating current mode with the square wave modulation in the potential range of $-0.4 \dots -1.8\text{ V}$, $W = 1000\text{ rpm}$, amplitude 40 mV , $\nu = 65\text{ Hz}$. CE was used as a working and an auxiliary electrode, and Ag, AgCl/KCl (sat) electrode type EVL-1M4 as a reference electrode.

Preparation of Standard Solution

Standard solution of Cefotaxime ($1 \cdot 10^{-2}\text{ mol L}^{-1}$): transfer 47.74 mg of cefotaxime into a 100 mL volu-



Scheme. The mechanism of Cefotaxime chemical transformations by means of potassium peroxomonosulfate in the acidic medium.

metric flask and dilute to the volume with double distilled water at 293 K.

Standard solution of hydrogenperoxomonosulfate ($2 \cdot 10^{-2} \text{ mol L}^{-1}$): transfer 68.30 mg of $2\text{KHSO}_5 \cdot \text{KHSO}_4 \cdot \text{K}_2\text{SO}_4$ into a 100 mL volumetric flask and dilute to the volume with double distilled water at 293 K. The solution of peroxomonosulfate was standardized iodometrically.

Procedure

Pipette aliquots of $0.5\text{--}5.00 \text{ mol L}^{-1}$ of the Cefotaxime test solutions studied into several 50 mL volumetric flask containing 3.5 mL of $0.02 \text{ mol L}^{-1} \text{ KHSO}_5$ solution and 1 mL of $0.1 \text{ mol L}^{-1} \text{ Na}_2\text{SO}_4$ solution (pH=3.3). Mix the content of each flask well, transfer the electrolyser and record the voltammetric curve.

Results and Discussion

Cefotaxime is rapidly oxidisable to its sulfoxide in a quantitative yield by the excess of potassium peroxomonosulfate (KHSO_5) (Scheme). Sulfoxide is reducible at the CE with the consumption of two electrons.

Voltammograms of Cefotaxime S-oxide solutions for different concentrations of cephalosporins were scanned. There are two peaks on the voltammetric curve (Fig. 1) of the Cefotaxime S-oxide solution: at -0.65 V (that corresponds to potassium peroxomonosulfate) and -1.3 V (the peak height was rising proportionally to CFTM concentrations increase). This peak was chosen as the analytical one.

The calibration curve method is simple in procedure, precise and provides a reliable way to calculate the uncertainty of the concentration calculated from the calibration curve (using the statistics of the least squares line fit to the data). These advantages allow to apply the calibration curve method for the assay results calculation (Fig. 2) given in Table.

Linearity

Linearity was studied over the small drug concentration range from $1 \cdot 10^{-4}$ to $1 \cdot 10^{-3} \text{ mol L}^{-1}$. The correlation coefficient $r = 0.999$ obtained for the regression

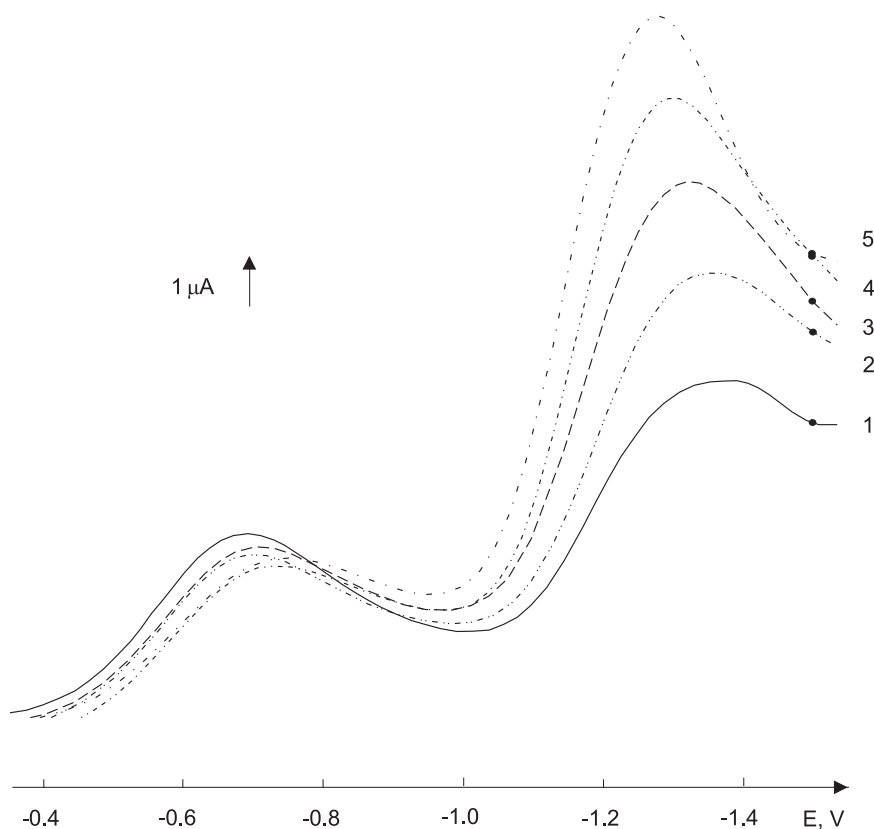


Fig. 1. The voltammetric curve of Cefotaxime S-oxide by means of potassium peroxomonosulfate, $c(\text{KHSO}_5) = 0.126 \cdot 10^{-2} \text{ mol L}^{-1}$, $c(\text{Cefotaxime})$: 1 = $2 \cdot 10^{-4}$, 2 = $4 \cdot 10^{-4}$, 3 = $6 \cdot 10^{-4}$, 4 = $8 \cdot 10^{-4}$, 5 = $1 \cdot 10^{-3} \text{ mol L}^{-1}$; $c(\text{Na}_2\text{SO}_4) = 0.02 \text{ mol L}^{-1}$; pH 3.3.

Table

Estimation of accuracy and precision of the initial rate method for determination of Cefotaxime powder for injection

Amount taken, (mol L ⁻¹ , 10 ⁻⁴)	Amount found (mol L ⁻¹ , 10 ⁻⁴)	Recovery (%±SD)	RSD (%)	δ (%)
3.96	3.92±0.10	98.52±0.61	1.75	-1.01
5.94	5.95±0.12	100.18±2.00	1.61	-0.17
9.90	9.84±0.15	99.37±1.51	1.22	-0.63

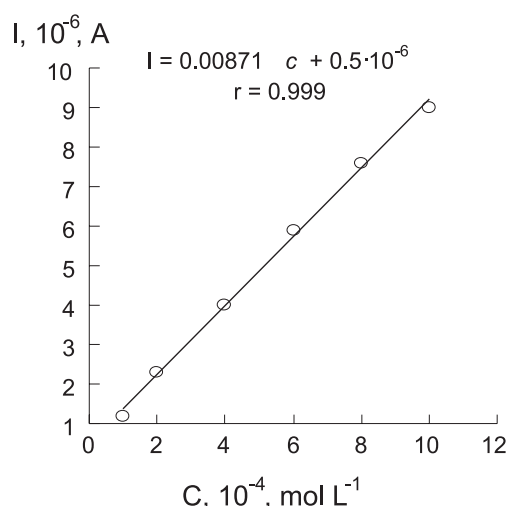


Fig. 2. The calibration graph for voltammetric determination of Cefotaxime in a pure substance. c (KHSO₅) = $0.126 \cdot 10^{-2}$ mol L⁻¹; c (Na₂SO₄) = 0.02 mol L⁻¹; pH 3.3.

line showed a good relationship between the I , μ A and the molar concentration of Cefotaxime (Fig. 2).

Accuracy, Precision Limit of Detection and Quantification

The validity of the method proposed was determined by performing recovery studies. Precision and accuracy were studied by analyzing five replicates of the sample solutions at three concentrations levels. The relative standard deviations calculated were all below 1.75% indicating the excellent precision of the procedure proposed.

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated based on the standard deviation of response and the slope of the calibration curve and expressed as:

$$\text{LOD} = 3 \times S_d/b, \text{ LOQ} = 10 \times S_d/b,$$

where: S_d is the standard deviation of the response; b is the slope of the calibration curve.

LOD = $1.2 \cdot 10^{-5}$ mol L⁻¹ and LOQ = $4 \cdot 10^{-5}$ mol L⁻¹.

The characteristics of the corresponding electrode reaction and its analytical parameters are presented and discussed. Optimum pH-ranges for determination of cefotaxime are given.

CONCLUSIONS

For the first time voltammetric determination of Cefotaxime by the reaction of S-oxidation by means of potassium peroxomonosulfate in a weak acidic medium has been optimized and proposed.

The calibration curve method can be easily applied for determination of Cefotaxime in the powder for injection that does not require the elaborate treatment and expensive materials. The method proposed is sensitive enough to enable determination of lower amounts of a drug; these advantages encourage the application of this method in the routine quality control of Cefotaxime in control and research laboratories. Finally, the method provides advantages of improving selectivity, it is quick and easy in performance.

REFERENCES

1. Al-Momani I.F. // *J. Pharm. Biomed. Anal.* – 2001. – Vol. 25. – P. 751-757.
2. Basaez L., Vanysek P. // *J. of Pharmac. and Biomed. Analysis.* – 1999. – Vol. 19. – P. 183-192.
3. Fogg A.G., Fayad N.M., Burgess C. // *Anal. Chim. Acta.* – 1979. – Vol. 110. – P. 107-115.
4. Fogg A.G., Fayad N.M., Burgess C., McGlynn A. // *Analyt. Chim. Acta.* – 1979. – Vol. 108. – P. 205-211.
5. <http://en.wikipedia.org/wiki/Cefotaxime>
6. Mrestani Ya., Neubert R.H.H., Härtl A. // *Analytica Chimica Acta.* – 1997. – Vol. 49. – P. 207-213.
7. Nuñez-Vergara L.J., Squella J.A., Silva M.M. // *Talanta.* – 1982. – Vol. 29. – P. 137-138.
8. Ogoverk B., Hudnik V., Gomiscek S. // *Frensenius Z. Anal. Chem.* – 1988. – Vol. 330. – P. 59-64.
9. Ogoverk B., Gomiscek S. // *J. Pharm. and Biomed. Anal.* – 1991. – Vol. 9. – P. 225-236.
10. Omar M., Adbelmageed O., Attia T. // *Talanta.* – 2009. – Vol. 77. – P. 1394-1404.
11. Saleh A., Askal H. // *Talanta.* – 2001. – Vol. 54. – P. 1205-1215.
12. Samanidou F., Ioannou A.S., Papadoyannis I.N. // *J. Chromat. B.* – 2003. – Vol. 788. – P. 147-158.
13. Samanidou V.F., Hapeshi E.A., Papadoyannis I.N. // *J. Chromat. B.* – 2004. – Vol. 809. – P. 175-182.
14. Zuman P., Kapetanovic V., Aleksic M. // *Anal. Lett.* – 2000. – Vol. 33. – P. 2821-2857.

ВОЛЬТАМПЕРОМЕТРИЧНЕ ВИЗНАЧЕННЯ ЦЕФОТАКСИМУ ЗА УЧАСТЮ КАЛІЮ ГІДРОГЕНОПЕРОКСОМОНОСУЛЬФАТУ

Ю.Ю.Лабузова

Ключові слова: вольтамперометрія; цефотаксим; калій гідрогенопероксомоносульфат

Робота присвячена розробці нової вольтамперометричної методики кількісного визначення цефотаксиму в порошку для приготування розчину для ін'єкцій за продуктом перексокислотного окиснення у вигляді S-оксиду в слабкокислому середовищі з використанням калій гідрогенопероксомоносульфату (KHSO_5) як аналітичного реагента. Були зняті вольтамперограми розчинів S-оксиду цефотаксиму для різних концентрацій цефалоспоринової солі. Катодна гілка вольтамперограми розчину S-оксиду цефотаксиму має два піки: при $-0,65\text{ В}$ (що відповідає калій гідрогенопероксомоносульфату) та $-1,3\text{ В}$ (висота піку змінювалася пропорційно зміні концентрації цефотаксиму), його обрано як аналітичний. Була показана можливість застосування методу калібрувального графіка. Лінійність була вивчена в діапазоні концентрацій від $1 \cdot 10^{-4}$ до $1 \cdot 10^{-3}$ моль/л. Коефіцієнт кореляції $r = 0,999$. Точність та відтворюваність вивчені шляхом п'ятикратного повторення аналізу для трьох концентрацій. Розраховане стандартне відхилення для усіх концентрацій було менше $1,75\%$, $\delta \leq 1,1\%$. Межа виявлення (МВ) і межа кількісного визначення (МКВ) були розраховані ($\text{МВ} = 1,2 \cdot 10^{-5}$ моль/л і $\text{МКВ} = 4 \cdot 10^{-5}$ моль/л). Встановлено, що запропонована вольтамперометрична методика для визначення лікарського препарату у низькій концентрації є досить чутливою, точною, правильною, відтворюваною і лінійною. Ці переваги дозволяють застосовувати її для кількісного визначення цефотаксиму в контрольно-дослідних лабораторіях.

ВОЛЬТАМПЕРОМЕТРИЧЕСКОЕ ОПРЕДЕЛЕНИЕ ЦЕФОТАКСИМА С УЧАСТИЕМ КАЛИЯ ГИДРОПЕРОКСОМОНОСУЛЬФАТА

Ю.Ю.Лабузова

Ключевые слова: вольтамперометрия; цефотаксим; калия гидропероксомоносульфат

Работа посвящена разработке новой вольтамперометрической методики количественного определения цефотаксима в порошке для приготовления раствора для инъекций по продукту перексокислотного окисления в виде S-оксида в слабкокислой среде с использованием гидропероксомоносульфата калия (KHSO_5) как аналитического реагента. Были сняты вольтамперограммы растворов S-оксида цефотаксима для различных концентраций цефалоспориновой соли. Катодная ветка вольтамперограммы раствора S-оксида цефотаксима имеет два пика: при $-0,65\text{ В}$ (что соответствует гидропероксомоносульфату калия) и $-1,3\text{ В}$ (высота пика менялась пропорционально изменению концентрации цефотаксима), который выбран как аналитический. Была показана возможность применения метода калибровочного графика. Линейность была изучена в диапазоне концентраций от $1 \cdot 10^{-4}$ до $1 \cdot 10^{-3}$ моль/л. Коэффициент корреляции $r = 0,999$. Точность и воспроизводимость изучены путем пятикратного повторения анализа для трех концентраций. Рассчитанное стандартное отклонение для всех концентраций было меньше $1,75\%$, $\delta \leq 1,1\%$. Предел обнаружения (ПО) и предел количественного определения (ПКО) были рассчитаны ($\text{ПО} = 1,2 \cdot 10^{-5}$ моль/л и $\text{ПКО} = 4 \cdot 10^{-5}$ моль/л). Установлено, что предложенная вольтамперометрическая методика для определения лекарственного препарата при низкой концентрации является чувствительной, точной, правильной, воспроизводимой и линейной. Эти преимущества позволяют применять ее для количественного определения цефотаксима в контрольно-исследовательских лабораториях.

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

UDC 615.225.2:54.062:543.544.5.068.7

VERIFICATION OF HPLC FOR THE QUANTITATIVE DETERMINATION METHOD OF NIFEDIPINE IN TABLETS

I.L.Komarytskyy, V.A.Khanin, N.Yu.Bevz, V.A.Georgiyants

National University of Pharmacy

Key words: nifedipine; verification; HPLC

Currently a great number of generic drugs have been registered in Ukraine. The advantage of generic drugs is a relatively low cost compared to innovative medicines since creation and registration of generics require less research and, consequently, less material costs for development, research and market penetration of a new drug. Because of creation of the second edition of SPhU and inclusion of articles on the finished products, we have set ourselves the goal to analyze the validation characteristics of the quantitative chromatographic determination of nifedipine in tablets and to verify the analytical procedure. Linearity of the method was determined in the range of 80-120% of the nominal concentration. Linearity of the method has been confirmed within the whole range of concentrations studied ($b = 1.0031$, $S_b = 0.0007816$, $a = -0.11$, $S_a = 0.07891$, $S_o = 0.03055$, $r = 1.0000$). It has been proven that the method suggested is characterized by sufficient convergence and accuracy over the entire range of concentrations ($\Delta_z = 0.06$, $\delta\% = 0.2$). The intermediate precision has been confirmed by the fact that the value of the relative confidence interval for five parallel measurements of one batch of the drug meets the acceptance criterion ($\Delta z = 0.22\% \leq 1.6\%$). Thus, according to the results of determining the validation characteristics of the methods for quantitative determination it has been substantiated and experimentally proven that this analytical procedure can be correctly reproduced, gives accurate results and is suitable for the analysis of nifedipine tablets. In the process of verification of the method for quantitative determination of nifedipine in tablets such validation characteristics of the chromatographic method as accuracy, linearity, precision, specificity and intermediate precision have been studied. The validation characteristics of the method do not exceed the critical value of error (1.6%) and are characterized by qualitative analytical indicators. This method can be correctly reproduced in the laboratory conditions.

Nifedipine is the main representative of calcium antagonists, derivatives of 1,4-dihydropyridine, which is widely used in medical practice. It blocks voltage-dependent calcium channels and prevents the penetration of calcium ions into smooth muscle cells of blood vessels. Nifedipine lowers blood pressure, improves the coronary blood flow, and exhibits the anti-anginal, hypolipidemic and antisclerotic effect. It is produced in the form of powder, solution for injections, capsules, tablets, ointment, drops and other medicinal forms [5].

The scientific literature describes methods for quantitative determination of nifedipine by cerimetric titration in the non-aqueous medium [8] and HPLC [13, 16, 17], voltammetry [12, 14], polarography [7] and UV spectrophotometry [1, 6, 10]. Besides, the method of highly sensitive kinetic determination of nifedipine using the luminol-persulfate chemiluminescence system is known [11]. For the quantitative determination of nifedipine USP37-NF32 [15] the use of the method of liquid chromatography is recommended.

Because of creation of the second edition of SPhU and inclusion of articles on the finished products, we have set ourselves the goal to analyze the validation characteristics of the quantitative chromatographic determination of nifedipine in tablets and to verify the analytical procedure.

Materials and Methods

When conducting the research the substance of nifedipine meeting the SPhU requirements was used.

The following analytical equipment was used: a 2695 chromatograph with a 2996 diode matrix detector of Waters Corp. firm, USA; XTerra RP18 column 250×4.6 mm with the particle size of 5 μ m, a ER-182 balance of AND company, Japan; glassware for measuring of class A.

We made a chromatogram of standard sample (SS) solution receiving from 2 to 6 chromatograms. The injection volume was 25 μ l. The relative standard deviation (RSD) was calculated for peak areas of the chromatograms obtained. Chromatography (n_0) was discontinued when reaching the values (RSD) specified in the requirements for suitability of the chromatographic system.

Chromatography was performed on a liquid chromatograph with a UV detector under the following conditions:

- mobile phase: acetonitrile for chromatography – methanol – water (25:25:50) degassed by ultrasound;
- detection: at the wavelength of 265 nm;
- the rate of the mobile phase: 1.0 ml/min.

Preparation of Test Solution

Place 5 tablets in a 50 ml volumetric flask, add 40 ml of the mobile phase, shake for 20 minutes, dilute to the volume with the mobile phase, mix and filter through a glass filter.

Preparation of SS solution

Place 20 mg of nifedipine USP RS in a 20 ml volumetric flask, add 10 ml of the mobile phase, shake to dissolve and dilute to the volume with the mobile phase, mix and filter through a glass filter.

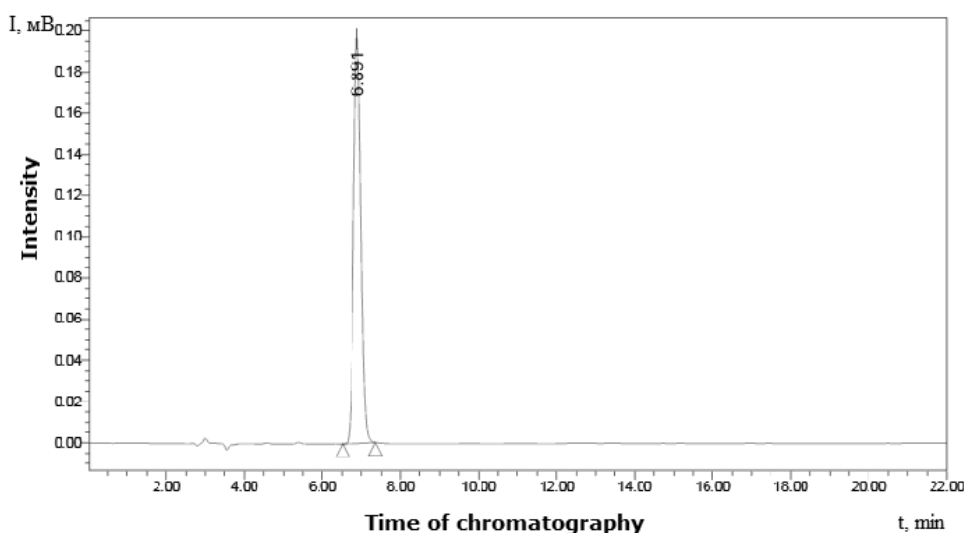


Fig. 1. The chromatogram of the Standard solution of nifedipine.

Before the main tests were validated, the presence of documents certifying the suitability of the equipment, raw material and chemicals was controlled.

Validation of the method was carried out in accordance with the requirements of the SPhU [2-4].

Results and Discussion

For elaboration of the method the chromatograms of the Standard solution of nifedipine (Fig. 1) and the Test solution of nifedipine (Fig. 2), as well as the dependence of the intensity peaks on the retention time were obtained and analysed.

The results of the analysis are considered reliable if the requirements of the System Suitability Test are performed. The chromatographic system is considered suitable if the following conditions are performed:

- the effectiveness of the chromatographic column calculated by the peak of nifedipine in the chromatogram of nifedipine SS must be not less than 4000 theoretical plates;
- the peak symmetry factor calculated by the peak in the chromatogram of nifedipine SS should be from 0.8 to 1.5;

- the relative standard deviation (RSD) for peak areas of nifedipine from the chromatogram obtained with the reference solution for 3 parallel measurements should be not less than 1.0%.

Our results of quantitative determination of nifedipine in the drug according to the method indicate its reproducibility.

Verification of the method for quantitative determination of nifedipine in tablets was performed by such validation characteristics as specificity, linearity, convergence, precision, accuracy and the intermediate precision.

To assess the accuracy of the sample preparation of the model solutions and standard sample solution the theoretical uncertainties of the analytical procedure that was $\Delta_{sp} = 1.07\% \leq B \cdot 0.32 = 1.6\%$ were calculated. Thus, the uncertainty of sample preparation calculated and analysis in general should provide sufficient accuracy.

Since high performance liquid chromatography (HPLC) used in the method is specific, then to prove that the method is specific it is sufficient to perform all requirements of the criteria for linearity, accuracy, precision and the intermediate precision.

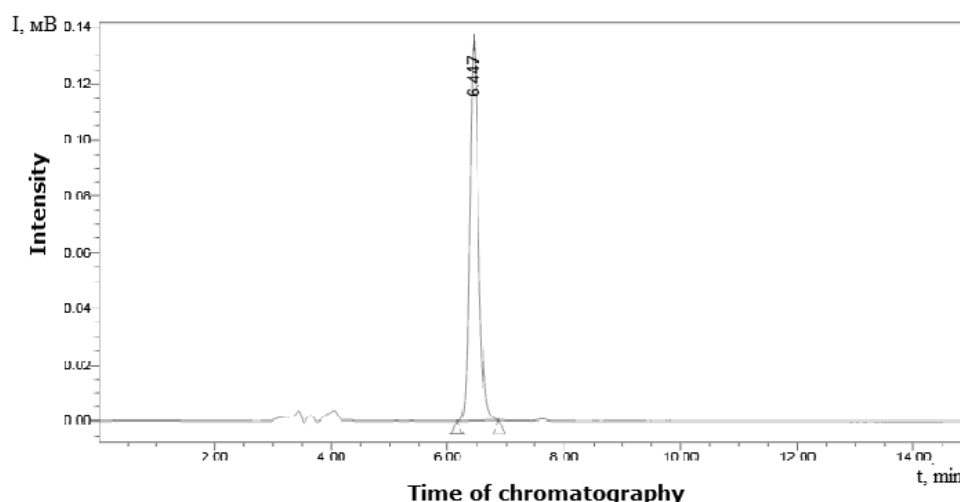


Fig. 2. The chromatogram of the Test solution of nifedipine.

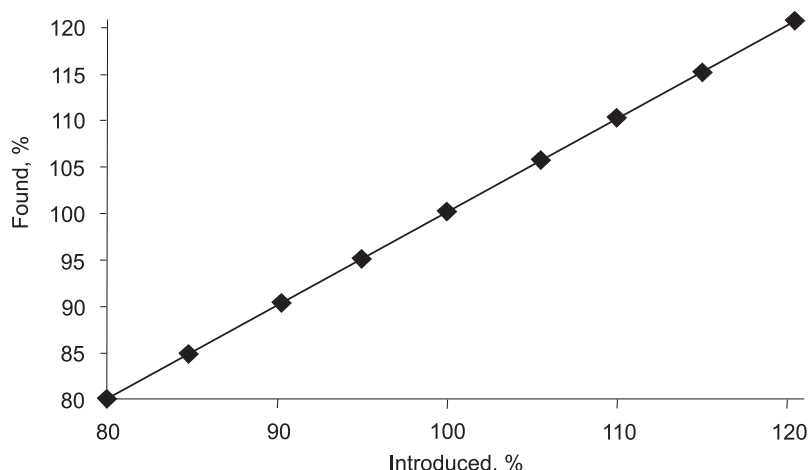


Fig. 3. The linear dependence of the peak area on the concentration of nifedipine in normalized coordinates.

Solutions for chromatography were prepared by the methods listed.

Evaluation of linearity was performed on the entire range of application of the method using the standard method. The study of dependence of absorbance on the concentration was conducted using 9 model solutions for analysis of the sample concentrations accurately weighed: 80, 85, 90, 95, 100, 105, 110, 115 and 120%.

The results obtained were statistically processed by the least squares method according to the requirements of the SPbU. The calibration graph was constructed in normalized coordinates (Fig. 3). For each of the nine test solutions the average values of the peak area (S_i) were calculated. The results obtained were processed by the least squares method for line $Y = b \times x + a$ (Fig. 3). The statistical quantities b , S_b , a , S_a , S_r (final standard

Table 1

Characteristics of the linear dependence

The slope of the linear relationship b	1.0031
S_b	0.0007816
The constant term of the linear dependence a	-0.11
S_a	0.07891
The residual standard deviation S_r	0.03055
The correlation coefficient method r	1.0000

deviation) calculated and r (correlation coefficient) are shown in Fig. 3 and in Table 1.

Requirements for the parameters of the linear dependence in this case are carried out within the whole range of the method application (80-120%).

Table 2

The results of analysis of model solutions and their statistical analysis

No. of the model solution	Introduced in % to the concentration of the reference solution ($X_i = C_i/C_{st}$ %)	Average peak areas (S_i) ($S_{st} = 1395946$)	Found in % to the concentration of the reference solution ($Y_i = S_i/S_{st}$ %)	Found in % to the introduced ($Z_i = Y_i/X_i$ %)
1	80.00	1117874	80.13	100.16
2	84.75	1223965	84.93	100.22
3	90.25	1309677	90.39	100.16
4	95.00	1346390	95.20	100.21
5	100.00	1375007	100.22	100.22
6	105.50	1465743	105.68	100.17
7	110.00	1535541	110.26	100.24
8	115.00	1612457	115.28	100.25
9	120.50	1669691	120.74	100.20
Mean, Z , %				100.20
Relative standard deviation, RSD_z , %				0.0324
Relative confidence interval, $\Delta Z(\%) = t(95\%, n-1) \times RSD_z = 1.860 \times RSD_z$, %				0.06
Critical value for convergence of the results ΔA_s , %				1.6
Systematic error $\delta = Z - 100 $				0.2
Criterion of the systematic error insignificance 1) $\delta\% \leq 1.03/3 = 0.34$ ($0.20 > 0.348$), if it is not satisfied 1), then $\delta\% \leq 0.32 \times 1.6 = 0.51\%$ ($0.20 < 0.51$)				is not satisfied satisfied
The overall conclusion of the procedure				correct

To measure and calculate the metrological evaluation of convergence and accuracy of the method three values of peak areas for the reference solution and 27 values of peak areas for model solutions were obtained. The actual values ($X_{i,act}$), the ratio of the average values of peak areas for each of 27 solutions were calculated to the mean peak area of the reference solution, the values $X_i = (C_i/C_{st}) \times 100\%$, $Y_i = (S_i/S_{st}) \times 100\%$, as well as the value $Z_i = (Y_i/X_i) \times 100\%$, the concentration found in % to the concentration introduced were determined. The calculation results are shown in Table 2.

To assess the intermediate precision the relative confidence interval for 5 parallel measurements of the quantitative content of substances, which should be less than the maximum permissible uncertainty analysis results ($\Delta_z \leq 1.6\%$), was used. Tests were carried out using one batch of the drug by different drug analysts on

the same chromatograph in different days using different measuring vessels.

Intermediate precision has been confirmed by the fact that the relative size of the confidence interval for five parallel measurements of one batch of the drug meets the acceptance criterion ($\Delta_z = 0.22\% \leq 1.6\%$).

CONCLUSIONS

1. In the process of verification of the method for quantitative determination of nifedipine in tablets such validation characteristics of the chromatographic method as accuracy, linearity, precision, specificity and intermediate precision have been studied.

2. The validation characteristics of the method do not exceed the critical value of error (1.6%) and are characterized by qualitative analytical indicators. This method can be correctly reproduced in the laboratory conditions, and independent of the excipients.

REFERENCES

1. Бугрова Е.А., Титова А.В., Арзамасцев А.П. // ХФЖ. – 2000. – №4. – С. 55-56.
2. Гризодуб А.И. // Фармаком. – 2006. – №1/2. – С. 34-44.
3. Державна фармакопея України / Державне підприємство «Науково-експертний фармакопейний центр». – Х.: РИПЕГ, 2001. – 556 с.
4. Державна фармакопея України / Державне підприємство «Науково-експертний фармакопейний центр». – Доп. 1. – Х.: РИПЕГ, 2004. – 520 с.
5. Компендиум 2009 – лекарственные препараты. В 2-х т. / Под ред. В.Н.Коваленко, А.П.Викторова. – К.: МОРИОН, 2009. – 2224 с.
6. Тимошик Ю.В., Петренко В.В. // Фарм. журн. – 2009. – №3. – С. 64-69.
7. Шаповалов В.А. // ЖАХ. – 2002. – №2. – С. 185-186.
8. British Pharmacopoeia. – London: The Stationary Office, 2009. – Vol. 1, 2. – 6481 p.
9. Ćwiczenia z chemii leków / Pod red. M.Gorczykowej, F.Zejca. – Krakov: Collegium Medium UJ, 1996. – 200 p.
10. Hemmateenejad B., Miri R., Kamali R. // J. Iran. Chem. Soc. – 2009. – №1. – P. 113-120.
11. He Shuhua, Lu Yi, He Deyong et al. // Chin. J. Anal. Chem. – 2004. – №4. – P. 474-476.
12. Madhusudana R.T., Jayarama R.S. // Anal. Lett. – 2004. – №10. – P. 2079-2098.
13. Niopas I., Daftsios A.C. // J. Pharm. and Biomed. Anal. – 2003. – №6. – P. 1213-1218.
14. Nuran O., Ceren Y., Suslu I. // J. Pharm. and Biomed. Anal. – 2002. – №3. – P. 573-582.
15. USP37-NF32 [Електронний ресурс]: – Режим доступу: <http://www.usp.org/usp-nf/pharmacopeial-forum>.
16. Vertzoni M.V., Reppas C., Archontaki H.A. // Anal. Chim. Acta. – 2006. – №573. – P. 298-304.
17. Yang Bingyi, Mo Jinyuan, Lai Rong et al. // Chin. J. Anal. Chem. – 2004. – №10. – P. 1304-1308.

ВЕРИФІКАЦІЯ ВЕРХ МЕТОДИКИ КІЛЬКІСНОГО ВИЗНАЧЕННЯ НІФЕДИПІНУ В ТАБЛЕТКАХ

І.Л.Комарицький, В.А.Ханін, Н.Ю.Бевз, В.А.Георгіянуц

Ключові слова: ніфедипін; верифікація; ВЕРХ

У теперішній час час в Україні зареєстровано велику кількість відтворених лікарських засобів (ЛЗ). Перевага відтвореного ЛЗ полягає у відносно невисокій вартості у порівнянні з оригінальним ЛЗ, оскільки його створення і реєстрація вимагають меншого обсягу досліджень і, відповідно, менших матеріальних витрат для розробки, дослідження та впровадження на ринок нового ЛЗ. У зв'язку зі створенням другого видання ДФУ і включенням до неї статей на готові лікарські засоби ми поставили собі за мету проаналізувати валідаційні характеристики методики кількісного хроматографічного визначення ніфедипіну в таблетках та провести її верифікацію. Лінійність методики визначали в межах 80-120% від номінальної концентрації. Лінійність методики підтверджується у всьому діапазоні досліджуваних концентрацій ($b = 1,0031$, $S_b = 0,0007816$, $a = -0,11$, $S_a = 0,07891$, $S_0 = 0,03055$, $r = 1,0000$). Доведено, що запропонована методика характеризується достатньою збіжністю і правильністю у всьому діапазоні концентрацій ($\Delta_z = 0,06$, $\delta\% = 0,2$). Внутрішньолабораторну прецизійність підтверджено тим, що величина відносного довірчого інтервалу для п'яти паралельних визначень однієї серії препарату задовольняє критерію прийнятності ($\Delta_z = 0,22\% \leq 1,6\%$). За

результатами визначення валідаційних характеристик методик кількісного визначення обґрунтовано та експериментально доведено, що дана аналітична методика може бути коректно відтворена, дає достовірні результати та придатна для аналізу таблеток ніфедипіну. У процесі верифікації методики кількісного визначення ніфедипіну в таблетках були вивчені валідаційні характеристики хроматографічної методики: правильність, лінійність, прецизійність, специфічність та внутрішньолaboratorна прецизійність. Валідаційні характеристики методики не перевищують критичного значення похибки (1,6%) і характеризуються якісними аналітичними показниками. Ця методика може бути коректно відтворена в умовах лабораторій з контролю якості лікарських засобів.

ВЕРИФИКАЦИЯ ВЭЖХ МЕТОДИКИ КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ НИФЕДИПИНА В ТАБЛЕТКАХ

И.Л.Комарицкий, В.А.Ханин, Н.Ю.Бевз, В.А.Георгиянц

Ключевые слова: нифедипин; верификация; ВЭЖХ

В настоящее время в Украине зарегистрировано большое количество воспроизведенных лекарственных средств (ЛС). Преимущество воспроизведенных ЛС заключается в относительно невысокой стоимости по сравнению с оригинальным ЛС, поскольку создание и регистрация воспроизведенного ЛС требуют меньшего объема исследований и, соответственно, меньших материальных затрат на разработку, исследование и внедрение на рынок нового ЛС. В связи с созданием второго издания ГФУ и включением в неё статей на готовые лекарственные средства мы поставили себе целью проанализировать валидационные характеристики методики количественного хроматографического определения нифедипина в таблетках и провести её верификацию. Линейность методики определяли в пределах 80-120% от номинальной концентрации. Линейность методики подтверждается во всем диапазоне исследуемых концентраций ($b = 1,0031$, $S_b = 0,0007816$, $a = -0,11$, $S_a = 0,07891$, $S_0 = 0,03055$, $r = 1,0000$). Доказано, что предложенная методика характеризуется достаточной сходимостью и правильностью на всем диапазоне концентраций ($\Delta_z = 0,06$, $\delta\% = 0,2$). Внутривлабораторная прецизионность подтверждена тем, что величина относительного доверительного интервала для пяти параллельных определений одной серии препарата удовлетворяет критерию приемлемости ($\Delta_z = 0,22\% \leq 1,6\%$). Таким образом, по результатам определения валидационных характеристик методик количественного определения обосновано и экспериментально доказано, что данная аналитическая методика может быть корректно воспроизведена, даёт достоверные результаты и пригодна для анализа таблеток нифедипина. В процессе верификации методики количественного определения нифедипина в таблетках были изучены валидационные характеристики хроматографической методики: правильность, линейность, прецизионность, специфичность и внутривлабораторная прецизионность. Валидационные характеристики методики не превышают критического значения погрешности (1,6%) и характеризуются качественными аналитическими показателями. Данная методика может быть корректно воспроизведена в условиях лабораторий контроля качества лекарственных средств.

Recommended by Doctor of Chemistry, professor M.Ye.Blazheyevskiy

UDC 543.062:543.422.7:615.262.1

THE ASSESSMENT OF THE METHOD FOR QUANTITATIVE DETERMINATION OF PREDNISOLONE IN THE OINTMENT BY THE REACTION WITH PHENYLHYDRAZINE

O.A.Ievtifieieva, K.I.Proskurina, O.M.Ganieva, V.T.Kirdan

National University of Pharmacy

Key words: photolorimetry; quantitative determination; prednisolone; validation; phenylhydrazine

Adaptation and validation of the analytical method of photolorimetric determination of prednisolone using the reaction with phenylhydrazine by the standard method have been carried out in the scientific research laboratory according to standard procedures as described in the State Pharmacopoeia of Ukraine. The main validation characteristics have been identified using the model solution with the standard substance. It has been found that the criteria of linearity and precision are observed in the range of concentrations of 80-120% (0.08-0.12 mg/ml in the test solution or 0.4-0.6% of prednisolone in the ointment), and they are statistically and practically insignificant ($a \leq S_a \times 1.86 = 4.7$, $\Delta a\% \leq 3.20 = 2.86$, $\delta \leq 1.0240 = 0.92$). The correct results obtained have allowed to test the method for determining prednisolone in the model extraction from "0.5% Prednisolone" ointment in the range of 80-120% of the nominal concentration. The study of robustness of the method performed has allowed to determine that it is necessary to follow a strict temperature range during the reaction to produce correct results ($60^\circ\text{C} \pm 1^\circ$). The amount of phenylhydrazine in the reagent may vary between 0.4-1.0 g/l. Criteria of linearity, precision and convergence for the model extraction of the ointment are also observed within the whole range of determination and are statistically and practically insignificant ($a \leq S_a \times 1.86 = 4.4$, $\Delta a\% \leq 3.20 = 2.71$, $\delta \leq 1.0240 = 1.00$). The method is acceptable for use in laboratories of the drug quality control and could be applied for determination of prednisolone in "0.5% Prednisolone" ointment with the hydrophobic base within the range of concentrations of 0.4-0.6%.

Prednisolone (Prednisolonum) – (11- β)-11,17,21-trihydroxypregna-1,4-diene-3,20-dione – is a dehydrated drug of the group of corticosteroids with an average therapeutic effectiveness that is widely used in medical practice in the form of injections, tablets, ointments, sprays, aerosols, creams, etc. However, injectable dosage forms and ointment are the most common. The latter is used to treat most of skin diseases [5].

For the quantitative determination of *prednisolone* in the substance the SPbU, EPh and a number of other pharmacopoeias recommend spectrophotometry as the variant of the absorbance method with preliminary purity control by HPLC [3, 6, 7, 10]. In soft dosage forms it is most frequently determined chromatographically (HPLC, TLC, etc.) [8]. The spectrophotometric method is uncommon because of the lower specificity, but it is much cheaper and easier to perform [9].

The aim of this research was the study and further adaptation of the method for quantitative determination of prednisolone in the ointment using the photolorimetric method by the reaction of phenylhydrazine.

Materials and Methods

For our research a pharmacopoeial standard sample of prednisolone PSS SPbU No.11/1-2143 (the shelf-life from 01/13/2014 to 01.2015, the content of prednisolone is 99.8%) and "0.5% Prednisolone" ointment Nizhpharm, RN UA/4949/01/01 batch 80414 were used.

In the experiment the following analytical equipment was used: a CPK-2 photolorimeter, cuvettes with the thickness of 10 mm, an AB 204 S/A METTLER

TOLEDO analytical balance, a TS-80 M-2 thermostat, reagents and measuring glassware of class A meeting the requirements of the SPbU.

The method of the quantitative spectrophotometric determination of prednisolone in the ointment by the reaction with phenylhydrazine:

Test solution: To the accurately weighed quantity of the ointment equivalent to 10.0 mg of prednisolone add 25 ml of 96% alcohol R. Heat on a water heater to dissolution of the base, then cool in ice. Filter the resulting mixture through a paper filter previously soaked in ethanol to a 100.0 ml volumetric flask.

Standard solution: Dissolve the accurately weighed quantity of the standard powder in 96% alcohol R preparing the solution with the accurate concentration of prednisolone equivalent to 0.1 mg/ml.

Sulfuric acid reagent (SAR): Prepare the solution of concentrated sulfuric acid, 96% alcohol R and purified water in the ratio of 4: 3: 3.

Modified phenylhydrazine sulfuric acid reagent (MPSAR): dissolve 65 mg of phenylhydrazine hydrochloride in 100 ml of SAR.

Procedure: Pipet 2.0 ml of the *Test solution* into each of two 50 ml conical flasks (identified as *Test solution* and *Blank Test solution*). Add 2.0 ml of the *Standard solution* into each of two 50 ml conical flasks (identified as *Standard solution* and *Blank Standard solution*). Pipet 2.0 ml of dehydrated alcohol into a 50 ml conical flask (identified as *Blank reagent*). Add 20.0 ml of SAR to the *Blank Test solution* and the *Blank Standard solu-*

Table 1

Metrological characteristics of the photocolorimetric method for quantitative determination of prednisolone by the reaction with phenylhydrazine using a standard solution of prednisolone

Parameters	Value	Criteria (for tolerances of $\pm 10\%$)	Conclusion
b	0.9430	–	–
S_b	0.0346	–	–
a	4.7	1) statistically acceptable value: $a \leq S_a \times 1.86$ 2) practically acceptable value: $a \leq 5.12$	satisfied satisfied
S_a	3.4882	–	–
RSD _o	1.3399	≤ 1.8070	satisfied
r	0.9998	≥ 0.9924	satisfied
Mean, Z%	99.08	–	–
Relative standard deviation, Sz%	1.54	–	–
Relative confidence interval $\Delta as\% = t(95\%,8) * Sz = 1.8595 * Sz =$	2.86	$\Delta as\% = 10.00 \times 0.32 = 3.20$	satisfied
Systematic error δ	0.92	$\delta \leq 1.0240$	satisfied

tion. Add 20.0 ml of MPSAR to the *Test solution*, *Standard solution* and *Blank reagent*. Place the flasks into a thermostat at $60^\circ\text{C} \pm 1^\circ$ for about 45 min, then cool in a chilled water cooler. Determine the absorbances of the solutions at the wavelength of the maximum absorbance at about 400 nm with a photocolorimeter against dehydrated alcohol. Calculate the amount of prednisolone in the ointment, in percents, by the formula:

$$C = \frac{(A - A_{sar} - A_{blank})}{(A_{st} - A_{sar/st} - A_{blank})} \cdot 100\%,$$

where: A – is the absorbance of the test solution; A_{sar} – is the absorbance of the blank test solution; A_{blank} – is the absorbance of the blank reagent; A_{st} – is the absorbance of the standard solution; $A_{sar/st}$ – is the absorbance of the blank standard solution.

The time of a single analysis takes approximately 1 hour 30 min.

Measurement of the optical density of the model solutions obtained was conducted three times removing the cuvette. Statistical analysis of the experimental data was carried out in accordance with Article of the SPhU “Statistical analysis of the results of chemical experiments” [2].

Because this method is validated for determination of prednisolone only in creams, and for ointments such methods are not available, it is impossible to predict accurately the effect of excipients and other conditions on the course of the reaction. Therefore, at first we investigated the basic validation characteristics of the method (linearity, precision, reproducibility) using the standard solution of prednisolone as a model.

Results and Discussion

It is known that during the interaction of prednisolone or other 17-oxysteroids with phenylhydrazine a coloured yellow compound is formed; its quantitative content can be determined by photocolorimetry [1]. This reaction is recommended by the USP for the quantitative analysis of corticosteroids in creams [11]. However, for ointments, in which other excipients are used, such methods are not available.

The assessment of *linearity* was performed by the standardized procedure within the whole range of determination (80–120% according to the SPhU). For this purpose 9 model solutions with accurate concentrations and the reference solution were used, their optical density was measured three times. The results obtained were statistically processed by least squares for the straight line $Y = b \cdot x + a$ as required by the SPhU. The calibration graph was plotted in the normalized coordinates [2, 4, 12]. The calculated statistical values b , S_b , a , S_a , RSD_o and r are given in Tab. 1. The assessment of *convergence* and *reproducibility* was conducted in parallel with determination of linearity by measuring the optical density of 9 model solutions three times.

Since the method of photocolorimetric determination of prednisolone by Porter-Zilber reaction using the standard solution of prednisolone as a model gives correct results, it has been decided to test it to determine the quantitative content of prednisolone in the ointment.

Before studying the validation characteristics of the method three samples of the ointment (100%) were analyzed. We received somewhat understated results than expected, so the study of robustness was conducted in more detail, namely the study of the effect of temperature and the amount of the reagent on the course of the reaction.

Table 2

The effect of temperature on the course of the photocolorimetric reaction of prednisolone with phenylhydrazine

Temperature, $^\circ\text{C}$	Optical density of the solution*	Optical density of the solution without excipients
20	0.13	0.05
40	0.32	0.15
60	0.46	0.25
100	0.21	0.13

*The average of 3 measurements taking into account the excipients.

Table 3

The effect of the amount of the reagent on the optical density

Amount of phenylhydrazine in 100 ml of the reagent / Optical density of the solution*				A_{average}	S_r	RSD, %	Δ , %	max δ , %
40 mg	60 mg	80 mg	100 mg					
0.510	0.505	0.503	0.510	0.507	0.0036	0.3559	0.76	1.024

*The average of 3 measurements taking into account the excipients.

Table 4

The study of metrological characteristics of the method for photocolometric determination of prednisolone by the reaction with phenylhydrazine

Parameters	Value	Criteria (for tolerances of $\pm 10\%$)	Conclusion
b	1.0552	–	–
S_b	0.0325	–	–
a	4.4	1) statistically acceptable value: $a \leq S_a \times 1.86$ 2) practically acceptable value: $a \leq 5.12$	satisfied satisfied
S_a	3.2746	–	–
RSD _o	1.2578	≤ 1.8070	satisfied
r	0.9998	≥ 0.9924	satisfied
Mean, Z%	101.00	–	–
Relative standard deviation, $S_z\%$	1.45	–	–
Relative confidence interval $\Delta as\% = t(95\%,8) * S_z = 1.8595 * S_z =$	2.71	$\Delta as\% = 10.00 \times 0.32 = 3.20$	satisfied
Systematic error δ	1.00	$\delta \leq 1.0240$	satisfied

The effect of temperature on the course of the photocolometric reaction. It has been found that the reaction is quite exacting to temperature fluctuations and can run with the formation of different products (Tab. 2). Therefore, we recommend to heat the reaction mixture and keeping it in a thermostat at $60^\circ \pm 1^\circ \text{C}$ in order to provide completeness of the reaction.

The effect of the amount of phenylhydrazine on the results of analysis. According to the literature to conduct the reaction studied the phenylhydrazine reagent should be used in the concentration of 0.065%. It has been found that the concentration range of phenylhydrazine within 0.04-0.1% allows to obtain the results of the required accuracy (Tab. 3).

Stability. According to the requirements of SPhU tests were carried out for an hour by measuring the optical density every 10 minutes (7 measurements). During this period of time the optical density did not change significantly and was 0.460 ($\Delta t = 0.54\% \leq 1.024\% = \text{max}\delta$) for the solution of the ointment taking into account the excipients.

The study of linearity was carried out by the standardized procedure measuring the optical density of 9 model solutions (containing prednisolone extracted from the ointment) with accurate concentrations and the re-

ference solution (containing prednisolone PSS) and three blank solutions. The method satisfies the requirements of linearity. The study of reproducibility and convergence also shows the correctness of the method (Tab. 4).

The values of LOD and LOQ were also calculated for the method; they were 9.31 and 31.03.

CONCLUSIONS

1. The main validation characteristics of the method of photocolometric determination of prednisolone by the reaction with phenylhydrazine have been determined for the model solution with the standard substance of prednisolone. It has been found that the method meets the validation criteria and can be tested to determine the ointment.

2. The parameters of linearity, reproducibility, convergence, stability and robustness for the method for quantitative determination of prednisolone in the ointment by the reaction with phenylhydrazine using the standard method have been determined. The correct results have been obtained in the range of 80-120% (corresponding to 0.4-0.6 g of prednisolone in the ointment).

3. The method can be used for quantitative determination of prednisolone in "0.5% Prednisolone" ointment in the domestic laboratories of the drug quality control.

REFERENCES

1. Гөрґз III. Количественный анализ стероидов / Пер. с англ. – М.: Мир, 1985. – 504 с.
2. Державна фармакопея України / Державне підприємство «Науково-експертний фармакопейний центр». – 1-е вид. – Х.: PIPEГ, 2001. – Доп. 1. – 2004. – 520 с.

3. Державна фармакопея України / Державне підприємство «Науково-експертний фармакопейний центр». – 1-е вид. – Х.: РІПЕГ, 2001. – Доп. 2. – 2008. – 620 с.
4. Евтифеева О.А., Георгиянц В.А. // Фармаком. – 2007. – №1. – С. 69-81.
5. Харкевич Д.А. Фармакология: Учебник. – 9-е изд., перераб., доп. и испр. – М.: ГЭОТАР-Медиа, 2006. – 736 с.
6. British Pharmacopoeia. – London. The Stationary Office, 2009. – Vol. 1-2. – 1952 p.
7. European Pharmacopoeia. – 6th ed. – Strasbourg: European Department for the Quality of Medicines, 2008. – 2416 p.
8. Makin H.L.J., Gower D.B. Steroid Analysis. – 2-nd ed. – New York, Springer, 2010. – 1213 p.
9. Singh D.K., Verma R. // Iranian J. of Pharmacol. & Therapeutics. – 2008. – Vol. 1, №7. – P. 61-65.
10. The International Pharmacopoeia. – 3-rd ed. – Geneva, 1981. – Vol. 2. – 342 p.
11. United States Pharmacopoeia 26. – United States Pharmacopoeial Convention, Rockville, MD, 2003.
12. Validation of analytical procedures: text and methodology : Q2(R1) / International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, November 2005, Geneva. – 12 p.

ОЦІНКА МЕТОДИКИ КІЛЬКІСНОГО ВИЗНАЧЕННЯ ПРЕДНІЗОЛОНУ В МАЗІ ЗА РЕАКЦІЮ З ФЕНІЛГІДРАЗИНОМ

О.А.Євтифеева, К.І.Проскуріна, О.М.Ганєва, В.Т.Кірдан

Ключові слова: фотоколориметрія; кількісний аналіз; преднізолон; валідація; фенілгідрозин

Проведено адаптацію та валідацію аналітичної методики фотоколориметричного визначення преднізолону за реакцією з фенілгідрозин методом стандарту в умовах науково-дослідної лабораторії за стандартними процедурами, описаними в Державній фармакопеї України. Визначені основні валідаційні характеристики при використанні стандартної субстанції для приготування модельного розчину та встановлено, що критерії лінійності та прецизійності виконуються в діапазоні концентрацій 80-120% (а саме 0,08-0,12 мг/мл в досліджуваному розчині або 0,4-0,6% преднізолону в мазі) та є статистично і практично незначущими ($a \leq S_a \times 1,86 = 4,7$, $\Delta a\% \leq 3,20 = 2,86$, $\delta \leq 1,0240 = 0,92$). Отримані коректні результати дозволили апробувати методику для визначення преднізолону в модельному вилученні з мазі «Преднізолон 0,5%» в діапазоні 80-120% від номінальної концентрації. Було проведено дослідження робастності методики та встановлено, що для отримання коректних результатів необхідно дотримуватися чіткого інтервалу температури при проведенні реакції ($60^\circ\text{C} \pm 1^\circ$). Кількість фенілгідрозину в реактиві при цьому може коливатися в межах 0,4-1,0 г/л. Критерії лінійності, прецизійності та збіжності для модельного вилучення з мазі також виконуються на всьому діапазоні визначення та є статистично і практично незначущими ($a \leq S_a \times 1,86 = 4,4$, $\Delta a\% \leq 3,20 = 2,71$, $\delta \leq 1,0240 = 1,00$). Методика є прийнятною для використання в лабораторіях контролю якості лікарських засобів і може бути запроваджена для визначення преднізолону в мазі «Преднізолон 0,5%» з гідрофобною основою в межах концентрацій 0,4-0,6%.

ОЦЕНКА МЕТОДИКИ КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ ПРЕДНИЗОЛОНА В МАЗИ ПО РЕАКЦИИ С ФЕНИЛГИДРАЗИНОМ

О.А.Евтифеева, К.И.Проскурина, О.М.Ганева, В.Т.Кирдан

Ключевые слова: фотоколориметрия; количественный анализ; преднизолон; валидация; фенилгидразин

Была проведена адаптация и валидация аналитической методики фотоколориметрического определения преднизолонa по реакции с фенилгидразином методом стандарта в условиях научно-исследовательской лаборатории согласно стандартных процедур, указанных в Государственной фармакопее Украины. Определены основные валидационные характеристики при использовании стандартной субстанции для приготовления модельного раствора и установлено, что критерии линейности и прецизионности соблюдаются в диапазоне концентраций 80-120% (а именно 0,08-0,12 мг/мл в исследуемом растворе или 0,4-0,6% преднизолонa в мази) и являются статистически и практически незначимыми ($a \leq S_a \times 1,86 = 4,7$, $\Delta a\% \leq 3,20 = 2,86$, $\delta \leq 1,0240 = 0,92$). Полученные корректные результаты позволили апробировать методику для определения преднизолонa в модельном извлечении из мази «Преднизолон 0,5%» в диапазоне 80-120% от номинальной концентрации. Проведенные исследования робастности методики позволили установить, что для получения корректных результатов необходимо придерживаться четкого интервала температуры при проведении реакции ($60^\circ\text{C} \pm 1^\circ$). Количество фенилгидразина в реактиве при этом может колебаться в пределах 0,4-1,0 г/л. Критерии линейности, прецизионности и правильности для модельного извлечения из мази также выполняются по всему диапазону определения и являются статистически и практически незначимыми ($a \leq S_a \times 1,86 = 4,4$, $\Delta a\% \leq 3,20 = 2,71$, $\delta \leq 1,0240 = 1,00$). Методика является приемлемой для использования в лабораториях контроля качества лекарственных средств и может применяться для определения преднизолонa в мази «Преднизолон 0,5%» с гидрофобной основой в пределах концентраций 0,4-0,6%.

Recommended by Doctor of Pharmacy, professor V.M.Kovalyov

UDC 577.118:581.45:582.681.71

THE STUDY OF THE ELEMENTAL COMPOSITION OF SUMMER SQUASH (*CUCURBITA PEPO* L).

Yu.A.Fedchenkova, I.I.Batyuchenko, O.P.Khvorost

National University of Pharmacy

Key words: summer squash; leaves; stalks; flowers; elemental composition

*Using the method of atomic emission spectrometry the qualitative composition and quantitative content of elements in the raw material (leaves, stalks, flowers) of summer squash (*Cucurbita pepo* L.) collected at different stages of vegetation have been determined. It has been found that leaves, stalks, flowers have identical elemental composition: 5 macro-, 10 micro- and 4 ultramicroelements concerning the qualitative aspect. For macroelements the regularity of increase in the content at the stages of vegetation was observed. There is the following regularity of the elemental content for the raw material: leaves and flowers – $K > Ca > Si > Mg > P > Na$, stalks – $K > Ca > Mg > Si > P > Na$. The highest content of potassium has been determined in all types of the raw material. The content of this element in stalk is 3340-5050 mg/kg, and it is higher compared to leaves by 1.2-1.5 times (2850-3354 mg/kg), compared to flowers – by 1.1-1.3 times (3000-4000 mg/kg). In leaves, stalks and flowers the considerable content of calcium (1350-1700 mg/kg, 1000-1576 mg/kg, 1007-1345 mg/kg, respectively), silicon (1299-1400 mg/kg, 470-500 mg/kg, 1000-1130 mg/kg, respectively) and magnesium (8000-1086 mg/kg, 759-800 mg/kg, 897-905 mg/kg, respectively) has been found. The content of such elements as molybdenum, cobalt and lead is lower than 0.03 mg/kg, arsenic, cadmium and mercury is lower than 0.01 mg/kg. The considerable content of such elements as calcium, silicon and magnesium in the types of the raw material studied indicates that leaves, stalks and flowers of summer squash can be a source for obtaining BAS.*

Nowadays despite a variety of synthetic medicines used in medical practice herbal drugs enjoy wide popularity. Our attention was drawn by summer squash (*Cucurbita pepo* L.), a melon plant. The plant is officinal, seeds are used as the raw material and an effective helminthagogue [7, 8]. By the power of the pharmacological action the seeds of summer squash yield to the raw material of a male fern, but they do not show toxic effect on the organism. It is also known that the pulp of summer squash increases the biliary excretion, improves the function of intestines in spastic colitis [2], intensifies the water-salt exchange, the renal filtration function, provides intensive release of chloride ions by the kidneys (promotes urination), has antioxidant properties, is an irreplaceable product in dietary food [10, 13].

The chemical composition of the officinal raw material is rather studied. The seeds contain fatty oil (about 20%), phytosterol cucurbitol, resinous substances, organic acids, vitamins of group B, ascorbic acid. The pulp contains sugars (glucose, fructose, sucrose), organic acids (mainly malic), carotene (up to 6 mg%), ascorbic (8-20 mg%), folic (14 mg%), pantothenic (0.4 mg%) and nicotinic (0.5 mg%) acids, vitamins B₁, B₂, B₆ (0.13 mg%), a significant amount of mineral substances (potassium, calcium, phosphorus, iron, copper, fluorine, zinc) [6, 9, 11]. Data on the chemical composition of stalks, leaves and flowers of summer squash is fragmentary. It is known that leaves contain up to 620 mg% of ascorbic acid, vitamins of group B, 438 mg% of potassium, 43 mg% of calcium, 38 mg% of magnesium and not less than 7 mi-

neral compounds, flowers have flavonoids and xanthophylls – cryptocapsin, zeaxanthin, flavoxanthin [5].

The mineral status of a plant is determined by the combined effect of different both necessary, and harmful mineral elements found in it. The Polish scientist Ya. Yancharsky arrived at the conclusion that the combination of three elements – magnesium, calcium and silicon was required for the correct metabolism. In turn, K. Riger proved that the main role of calcium in a human body was pH balancing (fight against "acidulation") and the help in killing cancer cells. Calcium also determines the selective permeability of cellular membranes, and its lack leads to increase in diffusion of various substances through membranes. Silicon increases the immunity, provides elasticity of vascular walls, has a positive effect on the protein biosynthesis, takes part in the connective tissue formation. Magnesium prevents from development of atherosclerosis, it is important in cardiovascular diseases, CNS excitation, diabetes, etc., is a part of chlorophyll molecules. The role of sodium in the life of a plant has not been finally found; this element promotes the creation of high osmotic pressure in cells.

The important indicator of the plant mineral status is the ratio of K^+/Na^+ , for it there are certain regular changes that are distinctive for different organs (root, leaves) of the plant. Researchers consider that the content of Na^+ at the level of 10 mg/kg in different plants is standard [12].

Based on the aforementioned the expansion of the source of the raw material for creation of new domestic medicines of the plant origin, the study of the chemical

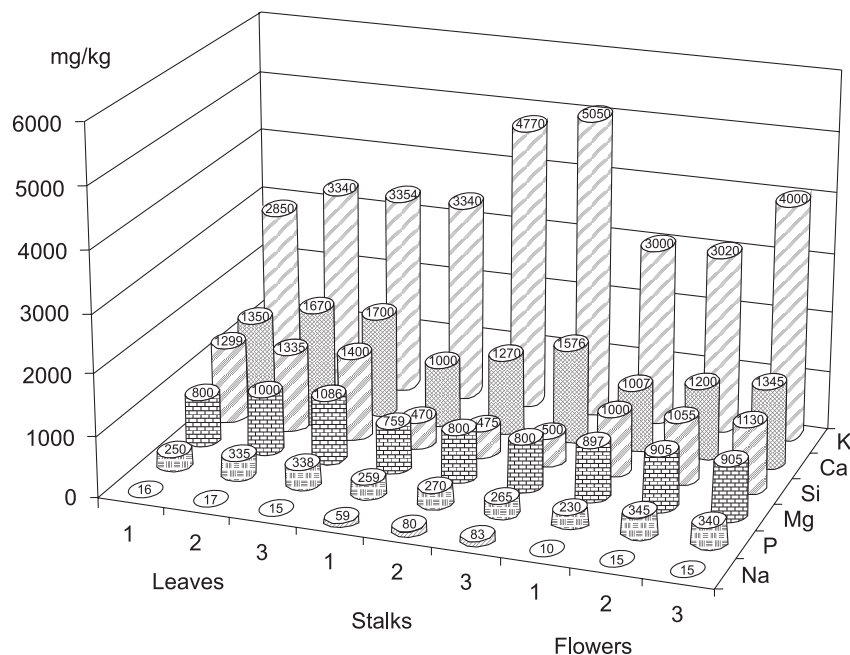


Fig. 1. Macroelemental composition of the raw material of summer squash. Stage 1 – the period of full opening of the leave blade (flowers were gathered as buds), stage 2 – the blossom period, stage 3 – the beginning of fructification.

composition of the raw material of summer squash, including the elemental one, is of current importance [3].

The aim of our research was to study the elemental composition of leaves, stalk and flowers of summer squash collected in different stages of vegetation.

Materials and Methods

The raw material (leaves, stalks, flowers) of summer squash, “Ukrainian polycarpous” sort [4], collected at different stages of vegetation in the Kharkiv region became the object of our research: stage 1 – the period of full deployment of a leaf blade (flowers were collected in the form of buds), stage 2 – the blossoming period, stage 3 – the period of the beginning of fructification. The study of the elemental composition was performed at the premises of the State Scientific

Institution “Institute for Single Crystals” of the NAS of Ukraine (Kharkov). The method of atomic emission spectrometry with photographic registration of results was used [1].

Results and Discussion

The diagram of the macroelemental composition of leaves, stalk and flowers of summer squash at different stages of vegetation is given in Fig. 1, the diagram of the microelemental composition is presented in Fig. 2.

It has been found that all types of the raw material – leaves, stalks and flowers concerning the qualitative aspect have the identical elemental composition presented by 19 elements. For macroelements the regularity of increase in the content at the stages of vegetation was observed (see Fig. 1). There is the following regulari-

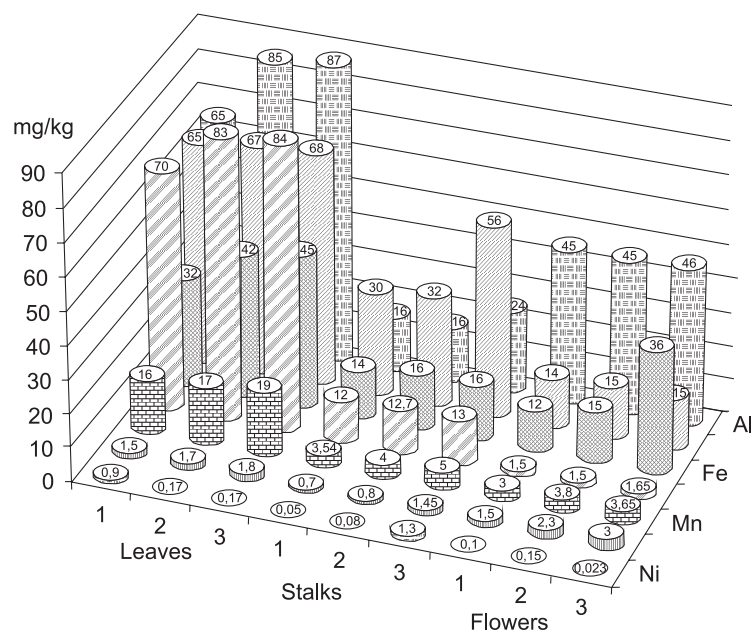


Fig. 2. Microelemental composition of the raw material of summer squash. Stage 1 – the period of full opening of the leave blade (flowers were gathered as buds), stage 2 – the blossom period, stage 3 – the beginning of fructification.

ty of the elemental content for the raw material: leaves and flowers – $K > Ca > Si > Mg > P > Na$, stalks – $K > Ca > Mg > Si > P > Na$. The content of such elements as molybdenum, cobalt and lead is lower than 0.03 mg/kg, arsenic, cadmium and mercury is lower than 0.01 mg/kg. The highest content in 3 types of the raw material is determined for potassium. The content of this element in stalk is 3340-5050 mg/kg, and it is higher compared to leaves by 1.2-1.5 times (2850-3354 mg/kg), compared to flowers – by 1.1-1.3 times (3000-4000 mg/kg). In leaves, stalks and flowers the considerable content of calcium (1350-1700 mg/kg, 1000-1576 mg/kg, 1007-1345 mg/kg, respectively), silicon (1299-1400 mg/kg, 470-500 mg/kg, 1000-1130 mg/kg, respectively) and magnesium (8000-1086 mg/kg, 759-800 mg/kg, 897-905 mg/kg, respectively) has been found.

The considerable content of such elements as calcium, silicon and magnesium in the types of the raw material studied indicates that leaves, stalks and flowers of summer squash can be a source for obtaining BAS.

CONCLUSIONS

1. Using the method of atomic emission spectrometry the qualitative composition and quantitative content of elements in the raw material of summer squash (*Cucurbita pepo* L.) collected at different stages of vegetation have been determined.

2. All types of the raw material – leaves, stalks, flowers in qualitative terms have the identical elemental composition. In each type of the raw material 5 macro-, 10 micro- and 4 ultramicroelements have been found. For all types of the raw material the highest content of potassium has been observed.

REFERENCES

1. Бурда Н.С., Журавель І.О., Кисличенко В.С., Демьохін В.Б. // Збірник наукових праць співробітників НМАПО ім. П.Л.Шупика. – 2010. – Вип. 19, кн. 3. – С. 586-589.
2. Вахрушев Я.М., Петрова Л.И. // Клиническая медицина. – 2004. – №10. – С. 49-51.
3. Государственная фармакопея СССР. Вып. 2. Общие методы анализа. Лекарственное растительное сырье / МЗ СССР. – 11-е изд. – М.: Медицина, 1989. – 400 с.
4. Колесник І.І., Заверталюк В.Ф. – Дніпропетровська дослідна станція ІОБ НААН. – Дніпропетровськ, 2011. – 10 с.
5. Ел. ресурс: <http://www.rusagroweb.ru/> – Овощеводство в России. Путательная ценность тыквы.
6. Azevedo-Meleiro C., Rodriguez-Amaya D. // J. Agric. Food Chem. – 2007. – Vol. 55, №10. – P. 4027-4033.
7. Caili F., Huan S., Quanhong L. // Plant Foods Hum. Nutr. – 2006. – №61. – P. 73-80.
8. Harchuk O.A., Mitina T.F., Kirilov A.F. et al. // Bul. IASM. Științevieți. Fiziologiası Biochimia Plantelor. – 2013. – №1 (319). – С. 71-78.
9. Kim M.Y., Kim E.J., Kim Y.N. et al. // Nutr. Res. Pract. – 2012. – Vol. 6, №1. – P. 21-27.
10. Saldeen K., Saldeen T. // Nutr. Res. – 2005. – №25. – P. 877-889.
11. Salwa E., Qiping Y., Ibrahim F. et al. // East African J. of Sci. – 2010. – Vol. 1, №1. – P. 77-89.
12. Taiz L., Zeiger E. Plant Physiology, 4th ed. Sinauer Associates. 2006. Inc. Sunderland, MA (QK711.2.T35 2006). – 705 p.
13. Wagner K.H., Kamal-Eldin A., Elmadfa I. // Ann. Nutr. Metab. – 2004. – №48. – P. 169-188.

ДОСЛІДЖЕННЯ ЕЛЕМЕНТНОГО СКЛАДУ СИРОВИНИ ГАРБУЗА ЗВИЧАЙНОГО CUCURBITA PEPO L.

Ю.А.Федченкова, І.І.Батюченко, О.П.Хворост

Ключові слова: гарбуз звичайний; листя; стебла; квітки; елементний склад

Методом атомно-емісійної спектроскопії визначено якісний склад та кількісний вміст елементів у сировині (листі, стеблах, квітках) гарбуза звичайного *Cucurbita pepo* L., зібраних у різні стадії вегетації. Встановлено, що листя, стебла, квітки в якісному плані мали однаковий елементний склад: 5 макро-, 10 мікро- та 4 ультрамікроелементів. Для макроелементів спостерігалася закономірність збільшення вмісту по стадіях вегетації. Має місце певна закономірність вмісту елементів для сировини: листя та квіток – $K > Ca > Si > Mg > P > Na$, стебла – $K > Ca > Mg > Si > P > Na$. Всім видам сировини був притаманний високий вміст калію. Так, для стебла він становив 3340-5050 мг/кг, що вище порівняно з листям в 1,2-1,5 рази (2850-3354 мг/кг), а порівняно із квітками – в 1,1-1,3 рази (3000-4000 мг/кг). В листі, стеблах та квітках теж у значних кількостях містилися кальцій (1350-1700 мг/кг, 1000-1576 мг/кг, 1007-1345 мг/кг, відповідно), силіцій (1299-1400 мг/кг, 470-500 мг/кг, 1000-1130 мг/кг відповідно) та магній (8000-1086 мг/кг, 759-800 мг/кг, 897-905 мг/кг відповідно). Вміст таких елементів як молібден, кобальт та плумбум нижчий за 0,03 мг/кг, вміст арсену, кадмію та ртуті – нижчий за 0,01 мг/кг. Значний вміст таких важливих елементів як кальцій, силіцій та магній у досліджуваних видах сировини свідчить про те, що листя, стебла та квітки гарбуза звичайного можуть стати джерелом отримання цих БАР.

**ИССЛЕДОВАНИЕ ЭЛЕМЕНТНОГО СОСТАВА СЫРЬЯ ТЫКВЫ ОБЫКНОВЕННОЙ
CUCURBITA PERO L.****Ю.А.Федченкова, И.И.Батюченко, О.П.Хворост****Ключевые слова:** тыква обыкновенная; листья; стебли; цветки; элементный состав

Методом атомно-эмиссионной спектроскопии определен качественный состав и количественное содержание элементов в сырье (листьях, стеблях, цветках) тыквы обыкновенной *Cucurbita perlo L.*, заготовленном в разные стадии вегетации. Установлено, что листья, стебли, цветки в качественном плане имели одинаковый элементный состав: 5 макро-, 10 микро- и 4 ультрамикроэлемента. Для макроэлементов наблюдалась закономерность увеличения содержания по стадиям вегетации. Наблюдается определенная закономерность содержания элементов для сырья: листьев и цветков – $K > Ca > Si > Mg > P > Na$, стеблей – $K > Ca > Mg > Si > P > Na$. Для всех видов сырья было характерно высокое содержание калия. Так, в стеблях оно составляло 3340-5050 мг/кг, что выше по сравнению с листьями в 1,2-1,5 раза (2850-3354 мг/кг), а по сравнению с цветками – в 1,1-1,3 раза (3000-4000 мг/кг). В листьях, стеблях и цветках тоже в значительном количестве содержался кальций (1350-1700 мг/кг, 1000-1576 мг/кг, 1007-1345 мг/кг соответственно), кремний (1299-1400 мг/кг, 470-500 мг/кг, 1000-1130 мг/кг соответственно) и магний (8000-1086 мг/кг, 759-800 мг/кг, 897-905 мг/кг, соответственно). Содержание таких элементов как молибден, кобальт и свинец ниже 0,03 мг/кг; мышьяка, кадмия и ртути – ниже 0,01 мг/кг. Значительное содержание таких важных элементов как кальций, кремний и магний в исследуемых видах сырья свидетельствует о том, что листья, стебли и цветки тыквы обыкновенной могут стать источником получения этих БАВ.

ТЕХНОЛОГІЯ ЛІКАРСЬКИХ ПРЕПАРАТІВ

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

UDC 615.451.124.014.23

THE STUDY OF THE PROPERTIES OF EMULSIONS BASED ON SEPIPLUS 400

T.Kovalyova, N.Polovko

National University of Pharmacy

Key words: emulsion; emulsifier; rheological properties; structural viscosity

Emulsifying properties of Sepiplus 400 and emulsions on its basis obtained by the method of cold emulsification have been studied. It has been found that the given emulsifier being in the concentration of 1-5% without adding co-emulsifiers and structure-forming agents allows to obtain stable emulsions within the range of the oil phase concentrations of 10-40%. Organoleptic, physicochemical, technological, structural and mechanical properties of the experimental samples have been investigated; they prove their belonging to the structured systems with satisfactory thixotropic properties, sensory characteristics and spread capacity. It has been proven that the mass fraction of the emulsifier, as well as the mass fraction of the oil contribute to the increased viscosity index of the samples examined. The results of the studies conducted are the evidence of the prospectivity of Sepiplus 400 in development of medicinal and cosmetic emulsions of various kinds of actions.

Currently polyanionic surface-active agents are widely used in manufacture of medicinal and cosmetic products. They are especially interesting as effective emulsion stabilizers [4, 9, 10].

One of the methods of emulsion stabilization is the use of micelles of block copolymers colloiddally dispersed in oil and having high emulsifying properties. Colloidal particles of the micelles accumulate at the oil-water interface, form mechanically firm structured films and thus stabilize the emulsion. The composition and the structure of such films are diverse and depend on the chemical structure of a stabilizer-emulsifier [6, 7].

One of the new promising emulsifiers for production of medicinal and cosmetic emulsions is the substance with a commercial name of Sepiplus 400 (Sepiplus™ 400, Seppic, France) and international unlicensed name of Polyacrylate – Polyisobutene – Polysorbate-20 (INCI).

Sepiplus 400 is a liquid polyanionic surface-active agent, which does not require any preliminary operations for its preparation: heating, neutralization, dispersion, etc. This is a ready-made emulsifying mixture, which is an invert emulsion where polymer chains are tightly convoluted in the internal aqueous phase. The mechanism of the emulsifying action of Sepiplus 400 is explained by the theory of “unwrapping drops”. The essence of this phenomenon consists in the phase inversion during which the polymer chains of Sepiplus 400 are unwrapping into the external aqueous phase, generating a stabilizing gel structure [5, 8].

The aim of our work was to study the emulsifying properties of Sepiplus 400, prepare the experimental samples of emulsions on its basis and study the organo-

leptic, structural and mechanical, as well as technological properties of the emulsions obtained.

Materials and Methods

The sets of experimental samples with different consistency were prepared by the method of cold emulsification with mineral and vegetable (corn) oil, their concentration varied between 5 and 50%. Sepiplus 400 was introduced as a monoemulsifier within the range of concentrations of 0.5-5% recommended by the manufacturer [2, 11]. The finished emulsions were tested by the following characteristics: appearance, colloidal and thermal stability, pH value, type of emulsion, some rheological properties, sensory characteristics (the rate of absorption, subjective sensations, stickiness, the absence of white traces when applying).

Colloidal and thermal stability were determined in accordance with the methodology of GOST “Cosmetic creams” [3]. The pH values of the experimental samples were determined potentiometrically in 10% water extraction of the cream with the help of a pH 150 MI pH-meter (Russia) according to the I-st ed., p.2.2.3. of the SPhU. Rheological studies were conducted with a BROOKFIELD HB DV-II PRO viscosimeter (USA) within the range of the shear rate from 0.1 sec⁻¹ to 150 sec⁻¹ (SC4-21 spindle for a chamber of 8.3 ml volume) at the temperature of 20 and 34°C. The type of emulsion was determined by the method of dilutions according to the SPhU [1].

Results and Discussion

The studies conducted allowed to select thermostable and colloiddally stable formulations, which were oil-in-water emulsions of different consistency – from

Table

The composition of the model samples of emulsions

No. of the sample	1	14	15	23	25	31	34	54	55	64
Mineral oil, %	0	–	10	15	–	15	20	–	30	40
Corn oil, %	–	10	–	–	15	–	–	30	–	–
Sepiplus 400, %	1.5	1.5	1.5	1	2	5	1.5	1.5	2	1.5
Purified water	up to 100.0									
Colloidal stability at 6000 rpm	+	+	+	+	+	+	+	+	+	+
Colloidal stability at 8000 rpm	+	+	+	+	+	+	+	+	+	+
Thermal stability	+	+	+	+	+	+	+	+	+	+
pH	6.18	6.20	6.18	6.12	6.25	6.5	6.22	6.21	6.38	6.25
Structural viscosity (mPa·s) at 20 rpm	3700	3420	3300	4700	7000	9100	4480	5680	6000	7940

Note: «+» – the emulsion is stable; «–» – the emulsion is unstable; n=5.

liquid to creamy one. The exception was Sample No. 1, which was an aqueous gel of a milk colour and creamy consistency, the oil was not introduced to it. The samples which do not pass the test of thermal and colloidal stability were removed from further studies. The model formulations selected were easy to apply, well spread on the skin and quickly absorbed, as well as created a comfortable sensation. It was also noted that the presence of corn oil in a number of emulsions considerably enhanced their tactile characteristics and eliminated the sense of stickiness when applying. The pH value of 10% solutions of the emulsions prepared varied between (6.12±0.05) and (6.45±0.05). The composition of some model samples is given in Table.

It was found that Sepiplus 400 was effective as a monoemulsifier in the concentration of 1.5-5%, and it allowed to obtain stable emulsions.

The rheological studies conducted showed the increase in viscosity of the samples when increasing the

emulsifier concentration (Fig. 1). Thus, Samples No. 23, 25 had the viscosity of 4700 and 7000 mPa·s (at 20 rpm and 20°C) with the concentration of the emulsifier of 1.5%, 2%; when increasing the emulsifier concentration up to 5% the viscosity considerably increased in Sample No. 31 and it was 9100 mPa·s.

When Sepiplus with the concentration of less than 1.5% was introduced into formulation 5 and 10% emulsions, destruction of the model samples occurred. It is explained most probably by the lack of polymer both for binding the particles of the internal phase and for creating colloidal protection by macromolecules.

At the same time Sample No. 31, which showed a positive test result for colloidal and thermal stability, decayed during the rheological studies breaking a general tendency of correlation of viscosity and the emulsifier quantity. In our opinion, it is connected with disturbance of the phase inversion behaviour as for this very process the dispersion medium–internal phase relation-

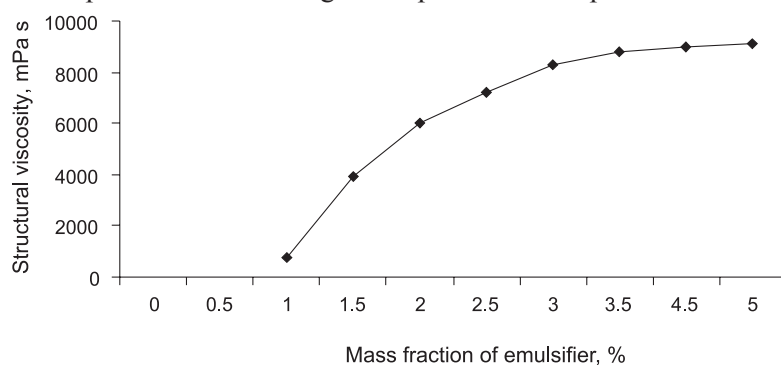


Fig. 1. Correlation between the structural viscosity of model formulations and the concentration of the emulsifier (at 20 rpm and 20°C).

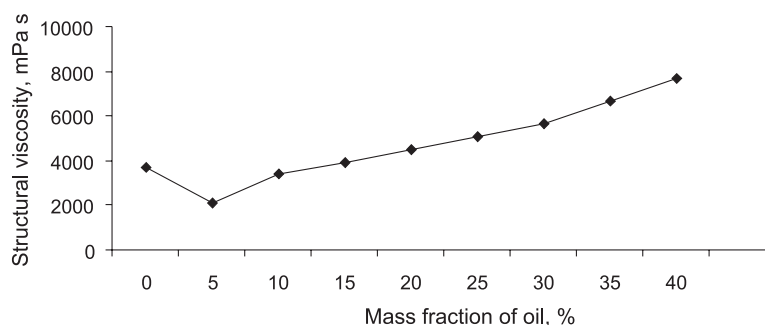


Fig. 2. Correlation between the structural viscosity of the model formulations and the oil concentration (at 20 rpm and 20°C).

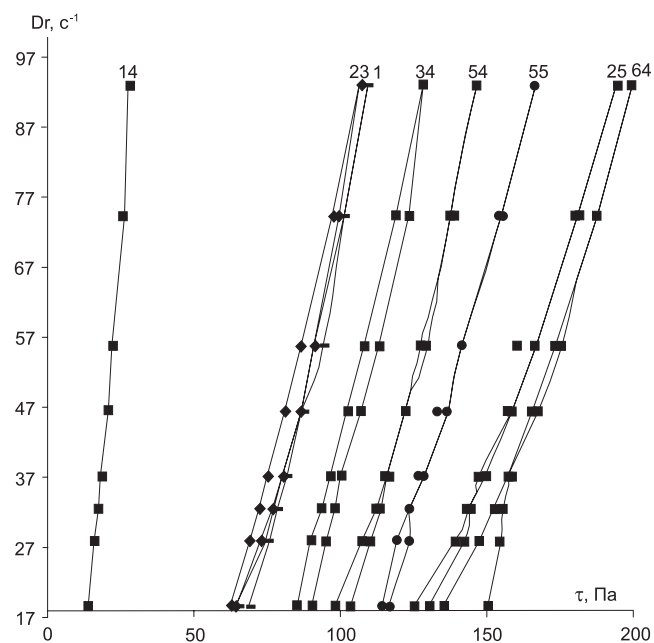


Fig. 3. The rheogram of the emulsion bases behaviour.

ship is important. It is known that there is a limiting (critical) relationship of volumes for each stabilizer in a certain concentration, exceeding of which results in formation of the invert emulsion. And the latter one is often unstable due to the low activity of the emulsifier in the changed conditions [9, 10].

Increase of the mass fraction of the oil phase also resulted in considerable increase of viscosity indices. Thus, the samples with the similar Sepiplus 400 content (1.5%), but with a different oil content (10, 20, 30 and 40%) showed the rising indices of viscosity: 3420 mPa·s, 4480 mPa·s, 5680 mPa·s and 7940 mPa·s, respectively (see Fig. 2). It should be also noted that corn oil slightly changed the viscosity of the bases obtained compared to the formulations with the similar content of liquid petrolatum. The sample without the oil showed a higher index of viscosity of 3700 mPa·s.

In order to estimate the viscous and plastic properties of model formulations their rheological parameters were determined. Based on the data obtained the complete rheograms of the model samples behaviour were plotted in such coordinates as the shear rate – shear stress (Fig. 3).

The rheograms built have a type of behaviour, which is typical for plastic systems, and a yield strength, which is within the following range of the shear stress: 14.7 Pa (Sample No.14) to 150.2 Pa (Sample No.64). The presence of the hysteresis loop in the rheograms confirms the presence of thixotropic properties of the samples studied.

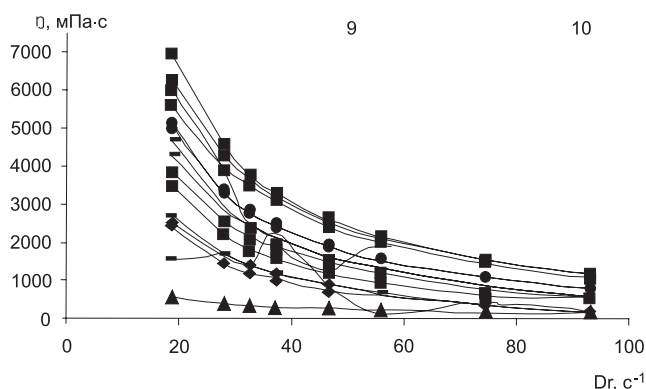


Fig. 4. The correlation between the structural viscosity of the samples studied and the shear rate.

Relatively low viscosity indices of the model samples are indicative of their light texture, which can quickly spread and absorb into the skin. This property can be taken as a basis for developing a line of cosmetic creams for skin care of various kinds of actions.

The next step of our work was the study of the correlation between the structural viscosity of the samples studied and the shear rate (Fig. 4).

The result of the study showed a gradual decrease of the indices of the structural viscosity as the shear rate increased. The 58-96 s⁻¹ range of the shear rate is notable for practically linear dependence of the structural viscosity on the shear rate, and it confirms the ability of emulsion systems to regenerate gradually after deformation.

CONCLUSIONS

The model samples of the emulsions with different consistency based on Polyacrylate – Polyisobutene – Polysorbate-20 have been developed.

It has been found that the given emulsifier being in the concentration of 1-5% without adding co-emulsifiers and structure-forming agents allows to obtain stable emulsions within the range of the oil phase concentrations of 10-40%.

Organoleptic, physicochemical, technological, structural and mechanical properties of the experimental samples have been investigated; they prove their belonging to the structured systems with satisfactory thixotropic properties, sensory characteristics and spread capacity.

It has been proven that the mass fraction of the emulsifier, as well as the mass fraction of the oil contribute to the increased viscosity index of the samples examined.

The results of the studies conducted are the evidence of the prospectivity of Sepiplus 400 in development of medicinal and cosmetic emulsions of various kinds of actions.

REFERENCES

1. Державна фармакопея України / Державне підприємство «Науково-експертний центр». – 1-е вид. – Х.: PIPEГ, 2001. – 556 с.
2. Ковалева Т.Н., Половко Н.П. Перспективы использования метода холодного эмульгирования в производстве эмульсионных кремов // Косметология: сегодня и завтра: Матер. науч.-практ. конф. за міжнар. участю. – Х.: НФаУ, 2013. – С. 68-69.
3. Креми косметичні. Загальні технічні умови: ДСТУ 4765:2007. – К.: Держспоживстандарт України, 2008. – 7 с.

4. Кутц Г., Фрисса С., Хеннинг С. и др. *Косметические кремы и эмульсии*. – М.: Косметика и медицина, 2004. – 272 с.
5. Лами Г., Борисенко А., Ляпунов Н., Безуглая Е. // *Промышленное обозрение*. – 2009. – №6 (17). – С. 54-55.
6. Aveyard R., Bernard P., Binks B.P., Clint J.H. // *Adv. Coll. Interface Sci.* – 2003. – Vol. 100. – P. 503-546.
7. Derkach S.R. // *Adv. Colloid. Interface Sci.* – 2009. – Vol. 151, №1-2. – P. 1-23.
8. Foudazi R., Masalova I., Malkin A.Ya. // *Colloid J.* – 2010. – Vol. 72. – P. 74-92.
9. Kragel J., Derkach S. // *Curr. Opinion in Colloid and Interface Sci.* – 2010. – Vol. 15, №4. – P. 246-255.
10. Miller R. *Interfacial Rheology. Progress in colloid and interface science* / Eds. R.Miller, L.Liggieri. – Leiden-Boston, Brill, 2009. – Vol. 1. – P. 142-156.
11. Raposo S. // *Pharm. Dev. Technol.* – 2013. – Vol. 19, №4. – P. 1-13.

ВИВЧЕННЯ ВЛАСТИВОСТЕЙ ЕМУЛЬСІЙ НА ОСНОВІ СЕПІПЛЮС 400

Т.М.Ковальова, Н.П.Половко

Ключові слова: емульсія; емульгатор; реологічні властивості; структурна в'язкість
Досліджені емульгуючі властивості Сепіплус 400 та емульсії на його основі, одержані методом холодного емульгування. Встановлено, що даний емульгатор у концентрації 1-5% без додавання співемульгаторів та структуроутворювачів дозволяє отримати стабільні емульсії в діапазоні концентрацій масляної фази 10-40%. Вивчені органолептичні, фізико-хімічні, технологічні, структурно-механічні властивості експериментальних зразків, які підтверджують їх приналежність до структурованих систем з задовільними тиксотропними властивостями, сенсорними характеристиками, намазуванням. Доведено, що масова частка емульгатора та масова частка масла сприяють підвищенню показника в'язкості досліджуваних зразків. Результати проведених досліджень свідчать про перспективність Сепіплус 400 для розробки лікарських та косметичних емульсій різного спрямування.

ИЗУЧЕНИЕ СВОЙСТВ ЭМУЛЬСИЙ НА ОСНОВЕ СЕПИПЛЮС 400

Т.Н.Ковалева, Н.П.Половко

Ключевые слова: эмульсия; эмульгатор; реологические свойства; структурная вязкость
Исследованы эмульгирующие свойства Сепиплюс 400 и эмульсии на его основе, полученные методом холодного эмульгирования. Установлено, что данный эмульгатор в концентрации 1-5% без добавления соэмульгаторов и структурообразователей позволяет получить стабильные эмульсии в диапазоне концентраций масляной фазы 10-40%. Изучены органолептические, физико-химические, технологические, структурно-механические свойства экспериментальных образцов, подтверждающие их принадлежность к структурированным системам с удовлетворительными тиксотропными свойствами, сенсорными характеристиками, намазываемостью. Доказано, что массовая доля эмульгатора и массовая доля масла способствуют повышению показателя вязкости исследуемых образцов. Результаты проведенных исследований свидетельствуют о перспективности Сепиплюс 400 в разработке лекарственных и косметических эмульсий различной направленности действия.

Recommended by Doctor of Medicine, professor N.I.Filimonova

UDC 615.281 : 547.913 : 661.155.8

SUBSTANTIATION FOR SELECTING A PRESERVATIVE WHEN DEVELOPING THE GEL WITH ESSENTIAL OILS FOR TREATING DISEASES OF THE UPPER RESPIRATORY TRACT

V.V.Pul-Luzan, O.P.Strilets, T.V.Martynuk

National University of Pharmacy

Key words: preservative; gel with essential oils; microbiological purity; method for evaluating the effectiveness of preservatives

Based on the results of the microbiological research the necessity of introduction of a preservative into the gel with essential oils developed for treating diseases of the upper respiratory tract has been proven. The microbiological research has been conducted according to the conventional method of the SPhU with the gel samples, in which the following preservatives were added: sodium benzoate, methylparaben, the complex preservative "Germaben", the complex of sodium benzoate with Nipagin in the ratio of 0.5:0.1. It has been found that all gel samples under study meet of the SPhU requirements for microbiological purity (according to the criterion "A" and "B"). While conducting the comparative analysis it has been determined that addition of the preservative complex of sodium benzoate with Nipagin in the ratio of 0.5:0.1 is the most effective.

At present there is a tendency to use topical drugs with a high aqueous phase content (creams water/oils, gels). However, these drugs are a favourable environment for microbial growth (cultures of *S. aureus*, *Ps. aeruginosa*) or spoiling of finished products (culture of mold fungi, yeast fungi of the *Candida* genus) [3, 5, 7].

As a result of the comprehensive research the composition of a gel with essential oils for treating diseases of the upper respiratory tract have been developed [6]. Due to the fact that the gel developed should be stable, including its microbiological purity value, at least for 2 years, it is necessary to study the microbiological purity of this gel and select the optimal preservative and its desired concentration [4, 5, 6].

It is known that when selecting a preservative the attention should be paid to the therapeutic effect of the drug, its area of application, interactability with other ingredients of the formulation, and its solubility. Based on the literature search in order to select an effective preservative (or their combination), which is able to provide the quality and safety of the gel with essential oils developed, we studied the antimicrobial activity of the experimental samples of gels with different preservatives. The following preservatives were selected for the studies: sodium benzoate, methylparaben (Nipagin, methyl ether of *n*-4-hydroxybenzoic acid), and the complex of sodium benzoate with Nipagin. The complex preservative "Germaben" was also studied, it included diazolidinyl urea, methylparaben, and propylparaben in propylene glycol [2, 8, 10].

Materials and Methods

The following gel samples were prepared: with 0.5% sodium benzoate (sample No. 1), with 0.1% methylparaben (sample No. 2), with 0.5% "Germaben" (sample

No. 3), with the complex of sodium benzoate and Nipagin (0.5% : 0.1%) (sample No. 4), and the sample without a preservative (sample No. 5).

During the studies the method for evaluating the effectiveness of antimicrobial preservatives given in the State Pharmacopoeia of Ukraine (SPhU) was used [4].

The culture media used in the study were standard manufactured by "Scientific Production Association Culture Media" (Mahachkala). The culture media were prepared according to the requirements of the manufacturer (the amount of the powder per litre, pH medium, autoclaving conditions, etc.). Each batch used in the experiment was tested for growth characteristics in compliance with regulations [1, 9].

Before the study there was a test conducted for conformity of the growth properties of the culture media (colony count grown when inoculating the microbial count). The culture media were inoculated with a small amount of the corresponding microbial test strains (10^2 co-

Table 1

The study of the microbiological purity of the gel samples

Samples of gels	Culture media and conditions Drug dilution in buffer 1 : 5	
	thioglycolic acid medium 28 days at 35°C	liquid Saburo medium 28 days at 25°C
No. 1	The growth is absent	The growth is absent
No. 2	—	—
No. 3	—	—
No. 4	—	—
No. 5	Microbial growth	—

Table 2

The study of the antibacterial activity of the gel samples with the preservative complex of sodium benzoate with Nipagin

Exposition	The SPhU requirements		The microbial count (CFU/ml), *Lg decrease		
	the bacterial count (CFU/ml), Lg decrease	the number of fungi (CFU/ml), Lg decrease	<i>Staphylococcus aureus</i> ATCC 6538	<i>Pseudomonas aeruginosa</i> ATCC 9027	<i>Candida albicans</i> ATCC 885/653
Microbial load	10 ⁶	10 ⁶	3.5×10 ⁵ *5.54	4.5×10 ⁵ *5.66	2.2×10 ⁵ *5.34
First inoculation Lg	–	–	4.9×10 ⁴ *0.85	5.1×10 ⁴ *0.96	5.2×10 ⁴ *0.63
2 days	2	–	3.3×10 ³ *2.02	2.7×10 ³ *2.23	1.3×10 ⁴ *1.23
7 days	3	–	1.2×10 ² *3.22	1.8×10 ² *3.41	2.2×10 ² *3.0
14 days	–	2	No isolation	No isolation	No isolation
28 days	No increase	No increase	No isolation	No isolation	No isolation

lony forming units per 1 ml of the medium – CFU/ml). The Saburo medium was plated with *Candida* yeast fungi, the nutrient agar – with *Pseudomonas aeruginosa*, and the egg yolk high salt agar culture medium – with *Staphylococcus aureus*. The thioglycolic acid medium was kept in thermostat at the temperature of 35°C for three days.

All bacterial cultures corresponded to taxonomic designation of the strain, and morphology of cultured colonies and cell morphology in microscopy were typical. The thioglycolic acid medium met the requirements for sterility – the growth of microorganisms was absent, the medium was transparent.

For the testing purpose a required number of suitable test microorganisms was added to the drug sample that was in the container, and the inoculated samples were stored at the corresponding temperature. At regular intervals the inoculated samples were sampled, and the microbial count was determined. The effectiveness of preservatives in the finished product is considered to be satisfactory if under conditions of the test while storing the inoculated samples at a given temperature during the given periods of time there is significant decrease or no increase in the microbial count depending on the requirements for the finished product.

Results and Discussion

According to Table 1 in 28 days of incubation while culturing in the thioglycolic acid medium the growth of microorganisms in the gel sample No. 5 was observed. Thus, it has been proven that addition of a preservative is required.

At the next step in order to select the optimal preservative (the samples mentioned above) the study was conducted for determining the microbiological purity. It has been found that sample No. 1 meets the criterion “B” in contrast to other samples meeting the criterion “A”.

As can be seen from Table 2, the preservative complex of sodium benzoate with Nipagin that meets the criterion “A” has the best preserving action.

The data given in Table 2 show that in 7 days of incubation the logarithm of the number of viable fungal cells was 3. There is no cells isolation in 14 and 28 days of incubation. In 2 days of incubation the logarithm of the microbial plate count was 2.02 and 2.23. On day 7 it was 3.22 and 3.41. On days 14th and 28 of incubation microorganisms were not recorded. The study of this sample has shown that it meets the criterion “A” in accordance with the SPhU requirements. Thus, the preservative complex of sodium benzoate with Nipagin has been selected for further studies.

CONCLUSIONS

1. As a result of the microbiological study it has been found that addition of a preservative to the gel with essential oils developed for treating diseases of the upper respiratory tract is mandatory.

2. It has been proven that all samples with the preservatives selected, namely methylparaben, the complex preservative “Germaben”, sodium benzoate, and the complex of sodium benzoate with Nipagin, meet the criteria “A” and “B” of the SPhU. The preservative complex of sodium benzoate with Nipagin that meets the criterion “A” has the best preserving action.

REFERENCES

1. Бактеріологічний контроль поживних середовищ. Інформаційний лист МОЗ України №05.4.1 / 1670. – К., 2001. – 10 с.
2. Беликов О.Е., Пучкова Т.В. Консерванты в косметике и средствах гигиены. – М.: Школа косметических химиков, 2003. – 250 с.
3. Гудзь О.В., Анисимова Ю.А., Яловенко Е.И. // Провизор. – 2000. – №12. – С. 38-39.
4. Державна фармакопея України / Державне підприємство «Науково-експертний фармакопейний центр». – 1-е вид. – Х.: PIPEF, 2001. – 301 с.

5. Лебединець О.В. Розробка складу та технології стоматологічного гелю комплексної дії: дис. ... канд. фармац. наук: 15.00.01. – X., 2012. – 146 с.
6. Павх О.І. Розробка складу та технології назальної мазі на основі рослинних настоек та ефірних олій: дис. ... канд. фармац. наук: 15.00.01. – Тернопіль, 2010. – 188 с.
7. Пуль В.В., Баранова І.І., Осолодченко Т.П. // Сучасні аспекти медицини і фармації: зб. тез Всеукр. наук.-практ. конф. молодих учених та студентів за міжнар. участю. – Запоріжжя, 15-16 травня, 2014. – С. 190.
8. Balk E.M., Zucker D.R., Engels E.A. et al. // J. Gen. Intern. Med. – 2001. – Vol. 16, №701. – P. 11.
9. Karlowsky J.A., Draghi D.C., Thornsberry C. et al. // Int. J. Antimicrob. Agents. – 2002. – Vol. 20, №178. – P. 76-85.
10. Rieger M.M., Banker G.S. – New York: Marcel Dekker. – 1989. – Vol. 10, №156. – P. 345.

ОБГРУНТУВАННЯ ВИБОРУ КОНСЕРВАНТА ПРИ РОЗРОБЦІ ГЕЛЮ З ЕФІРНИМИ ОЛІЯМИ ДЛЯ ЛІКУВАННЯ ЗАХВОРЮВАНЬ ВЕРХНІХ ДИХАЛЬНИХ ШЛЯХІВ

В.В.Пуль-Лузан, О.П.Стрілець, Т.В.Мартинюк

Ключові слова: консервант; гель з ефірними оліями; мікробіологічна чистота; методика оцінки ефективності консервантів

На теперішній час відмічається тенденція до використання лікарських препаратів зовнішньої дії з високим вмістом водної фази (креми о/в, гелі). Однак дані засоби є сприятливим середовищем для розвитку мікроорганізмів (культури *S. aureus*, *Ps. aeruginosa*) або псування готової продукції. У результаті проведених комплексних досліджень нами було розроблено склад гелю з ефірними оліями для лікування захворювань верхніх дихальних шляхів. У зв'язку з тим, що розроблений гель повинен бути стабільним, у тому числі за показником мікробіологічної чистоти впродовж не менше 2-х років, потрібне вивчення мікробіологічної чистоти розробленого гелю та вибір при необхідності консерванту та його оптимальної концентрації. Відомо, що при виборі консерванту слід обов'язково звертати увагу на терапевтичну дію препарату, ділянку його нанесення, можливість взаємодії з іншими інгредієнтами рецептури, його розчинність. З урахуванням проведеного літературного пошуку з метою вибору ефективного консерванту (або їх комбінації), який би гарантував якість і безпечність розробленого гелю з ефірними оліями, нами було вивчено антимікробну активність експериментальних зразків гелів з різними консервантами. Для досліджень було обрано такі консерванти: натрію бензоат, метилпарабен (ніпагін, метиловий ефір *p*-гідроксibenзойної кислоти), а також комплекс натрію бензоату з ніпагіном. Також досліджувався комплексний консервант «Гермабен», до складу якого входять діазолідинілсечовина, метилпарабен, пропілпарабен у пропіленгліколі. Проведені дослідження показали, що комплекс консервантів натрію бензоату з ніпагіном має найкращу консервуючу дію та відповідає критерію «А» згідно з вимогами ДФУ.

ОБОСНОВАНИЕ ВЫБОРА КОНСЕРВАНТА ПРИ РАЗРАБОТКЕ ГЕЛЯ С ЭФИРНЫМИ МАСЛАМИ ДЛЯ ЛЕЧЕНИЯ ЗАБОЛЕВАНИЙ ВЕРХНИХ ДЫХАТЕЛЬНЫХ ПУТЕЙ

В.В.Пуль-Лузан, О.П.Стрелец, Т.В.Мартинюк

Ключевые слова: консервант; гель с эфирными маслами; микробиологическая чистота; методика оценки эффективности консервантов

В настоящее время отмечается тенденция к использованию лекарственных препаратов наружного действия с высоким содержанием водной фазы (кремы м/в, гели). Однако данные средства являются благоприятной средой для развития микроорганизмов (культуры *S. aureus*, *Ps. Aeruginosa*) или порчи готовой продукции (культуры плесневых грибов, дрожжеподобные грибы рода *Candida*). В результате проведенных комплексных исследований нами был разработан состав геля с эфирными маслами для лечения заболеваний верхних дыхательных путей. В связи с тем, что разработанный гель должен быть стабильным, в том числе по показателю микробиологической чистоты на протяжении не менее 2-х лет, необходимы изучение микробиологической чистоты разработанного геля и выбор оптимального консерванта и его концентрации. Известно, что при выборе консерванта необходимо обращать внимание на терапевтическое действие препарата, участок его нанесения, возможность взаимодействия с другими ингредиентами рецептуры, его растворимость. Учитывая проведенный литературный поиск с целью выбора эффективного консерванта (или их комбинации), который бы гарантировал качество и безопасность разработанного геля с эфирными маслами, нами была изучена антимикробная активность экспериментальных образцов гелей с различными консервантами. Для исследований были выбраны такие консерванты: натрия бензоат, метилпарабен (нипагин, метиловый эфир *p*-гидроксibenзойной кислоты), а также комплекс натрия бензоата с нипагином. Также исследовался комплексный консервант «Гермабен», в состав которого входят диазолидинилмочевина, метилпарабен, пропилпарабен в пропиленгликоле. Проведенные исследования показали, что комплекс консервантов натрия бензоата с нипагином оказывает наилучшее консервирующее действие и отвечает критерию «А» соответственно требованиям ГФУ.

Recommended by Doctor of Pharmacy, professor O.G.Bashura

UDC 687.5: 687,552: 661.185

DEVELOPMENT OF THE SHAMPOO FOR CHILDREN

L.S.Petrovskaya, O.V.Zhuk, I.I.Baranova

National University of Pharmacy

Key words: shampoo for children; detergent; foam cleaning base; foaming ability

Children, especially infants, have a sensitive and very vulnerable skin. Cosmetics for children supports the protective functions of the skin, making it less susceptible to a variety of stimuli. That is why children from the first days of life need other, safer skin care cosmetics than adults, it should not have the irritating action. In order to develop a foam cleaning product (shampoo) for children we have chosen a number of modern detergents of anionic, non-anionic and amphoteric character, such as 28% disodium laureth sulfosuccinate ("Euronaat LS 3", Disodium Laureth-3-Sulfosuccinate, "EOS", Belgium); cocamidopropyl betaine ("Cocamidopropyl Betain", "KAO", Japan); coco glucoside and glyceryl oleate ("Lamesoft PO 65", Coco Glucoside (and) Glyceryl Oleate, "BASF (ex-Cognis)", Germany); PEG-7 glyceryl cocoate ("Neopal LIS 80", PEG-7 Glyceryl Cocoate, "Industria Chimica Panzeri", Italy). Lactic acid was used as a pH adjustor. Based on these substances the foam cleaning bases in various concentrations of detergents have been prepared. The quality (physicochemical properties) of the foam cleaning bases developed has been assessed according to the current normative documents of Ukraine. At the first stage of the research it was determined that the use of "Euronaat LS 3" was irrational because it did not provide the required foam formation, hence, it was necessary to add other detergents. At the next stage the bases with addition of detergents with the low irritating effect, such as "Cocamidopropyl Betaine", "Lamesoft PO 65", "Neopal LIS 80", were studied to improve the physicochemical and consumer properties. It has been found that foam cleaning bases with additionally selected non-ionic and amphoteric detergents improved the properties of the foam cleaning base developed, namely the level and stability of the foam and its consumer properties increased. It should be noted that the bases developed were stable at the pH value selected (5.5-6.5).

The baby skin like all other organs develops gradually. And though at first glance it differs little from the adult's skin, it may take a lot of time before the baby's skin can fully perform all of its functions. Functions of the skin are diverse, but the main function is protective. This function in children is poorly expressed as evidenced by the easily vulnerable skin, frequent infections due to insufficient keratinization of the stratum corneum and its thinness, immaturity of the local immunity and intense blood supply. These peculiarities make the baby skin tender and prone to inflammation, in particular with poor care; that is why the skin care in children assumes daily hygiene and water procedures. Children, as well as adults, also need hair care. It is known that the skin of the baby's head does not yet contain special natural protective film, which adults have. That is why microbes and other harmful substances penetrate through the children skin faster than in adults. It is obvious that the products for the baby skin care must meet other criteria than the products for adults. Since the skin of a normal healthy child is capable to cope with its functions, the question of safety of cosmetic products should be considered first [4, 7, 10, 11].

The aim of our work is to create a foam cleaning base with safe detergents and based on it to develop a modern foam-cleaning agent for children.

Experimental Part

The study subjects were a number of modern detergents of anionic, amphoteric and non-ionic nature such as 28% disodium laureth sulfosuccinate ("Euronaat LS 3",

Disodium Laureth-3-Sulfosuccinate, "EOS", Belgium); cocamidopropyl betaine ("Cocamidopropyl Betain", "KAO", Japan); coco glucoside and glyceryl oleate ("Lamesoft PO 65", Coco Glucoside (and) Glyceryl Oleate, "BASF (ex-Cognis)", Germany); PEG-7 glyceryl cocoate ("Neopal LIS 80", PEG-7 Glyceryl Cocoate, "Industria Chimica Panzeri", Italy). Lactic acid was used as a pH adjustor. Based on these substances the foam cleaning bases were developed in various concentrations of detergents [2, 12-16].

The quality of the bases developed together with the Pharmaceutical Research Centre "Beauty alliance", Kyiv was assessed according to the following indicators: appearance, organoleptic characteristics (colour, odour), determination of the pH value, foaming ability (foam number, foam stability). These indicators were considered for the qualitative assessment of modern foam cleaning agents according to DSTU4315: 2004 "Cosmetic products for cleaning skin and hair" and TU U24.5-31640335-002: 2007 "Products for skin care and cleaning".

The study of rheological indicators was carried out on a BROOKFIELD DV-II + PRO viscosimeter (USA) using a rotary adapter with the system of coaxial cylinders. The coaxial cylindrical geometry of the viscosimeter consists of a cylindrical spindle and a cylindrical camera, which provide precise measurement control of rheological parameters of Newtonian fluids [1, 3, 6, 8, 9].

The pH value level of the samples under study was determined by potentiometry (SPhU 1.2, 2.2.3) using a "pH Meter Metrohm 744" device (Germany).

Table 1

The study of the foaming ability
of the base
of disodium laureth sulfosuccinate

The concentration of disodium laureth sulfosuccinate, %	Foaming ability	
	Foam number, mm	Foam stability, c.u.
5.0	34.0	0.91
10.0	54.0	0.96
15.0	64.0	0.94

Results and Discussion

At the first stage the experimental samples of water solution of disodium laureth sulfosuccinate with the concentration of 5%, 10% and 15% were prepared. They were prepared at the room temperature at low speed of the mixer (20 rev/min) to prevent the formation of air bubbles. After complete dissolution of the detergent clear homogeneous liquid solutions without a specific odour were obtained. The pH value of these solutions was within 6.71-6.80, the standard rate of foam cleaning agents for children aged from 3 and older – pH 5.5 [5]. The pH was adjusted to the required value with lactic acid. The foaming ability and the foam stability were studied. According to DSTU 4315: 2004 the standard rate of foam number is not less than 145.0 mm, and the foam stability is 0.8-1.0 conditional units (c.u.).

As seen from the results (Table 1), the foam number was only 34.0 mm at 5% concentration of surfactants, and the foam stability index had a high value – 0.91 c.u. Both the foam number and the foam stability increased significantly in 10% solution of the surfactant. When the concentration increased up to 15%, the foam number reached the maximum and became 64.0 mm; at the same time, the foam stability was 0.94 c.u. Therefore, the conclusions can be made that this component should be used in the concentration of 10% since the solution has a relatively high value of the foam number and the highest value of the stability foam indicator.

It is irrational to use disodium laureth sulfosuccinate alone because it does not provide the required value of foam number according to DSTU 4315:2004. Based on the above experiment it has been proven that it is reasonable to introduce additional surfactants. Cocamidopropyl betaine was used as an additional surfactant. This is the most common surfactant used not only in development of products for children, but also in skin care products for adults. It is used to improve the foam level, stabilization of formulations, promotes cleansing properties, and in combination with some surfactants may contribute to the system's thickening. Its maximum recommended concentration is 12%.

At the next stage the required amount of 35% solution of cocamidopropyl betaine was added to 10% solution of disodium laureth sulfosuccinate at the room temperature. Cocamidopropyl betaine was completely dissolved within 5 min at the constant work of a mixer. It was added in the concentrations of 4%, 8% and 12%. The pH of solutions obtained was adjusted to 6.82 – 6.88

Table 2

The study of the foaming ability
of the base of disodium laureth sulfosuccinate
and 35% solution of cocamidopropyl betaine

The concentration of 35% solution of cocamidopropyl betaine, %	Foaming ability	
	Foam number, mm	Foam stability, c.u.
4.0	56.0	0.91
8.0	85.0	0.96
12.0	90.0	0.95

with lactic acid. As a result, transparent odourless liquid solutions were obtained.

As we can see from the research results (Table 2), the foam number increased in all samples, and foam stability of 4% and 12% solutions decreased, but the stability of 8% solution remained unchanged. Thus, the effective concentration of 35% solution of cocamidopropyl betaine is 8%, so the solution has a high foam number and the highest indicator of the foam stability.

The next step in development of the base is to stabilize the level of the foam. Coco glucoside / glyceryl oleate has been chosen as a foam stabilizer and a degreasing agent, its recommended concentrations for children care products is from 1% to 10%.

Since this surfactant is a concentrated substance, which is poorly soluble in cold water, 10% solution of disodium laureth sulfosuccinate with addition of 8% of 35% solution of cocamidopropyl betaine to it should be boiled previously to the temperature of 40-42°C. When rotating the mixer with 40 rev/min the amount of coco glucoside/glyceryl oleate required was added to the solution. This detergent was completely dissolved at the constant work of the mixer for 5-7 min. Thus, simultaneously five samples with the concentrations of 1, 2, 3, 4, 5% were prepared. As a result, a light yellow transparent odourless solution was obtained. The pH of the solutions obtained was 6.78-6.50; therefore, the pH value was adjusted to the desired value of 5.5 with lactic acid.

As can be seen from the results (Table 3), this component greatly improves the stability of the foam, but at higher concentrations coco glucoside/glyceryl oleate reduces the foam number more than twice from 123 mm to 66 mm because the foam becomes fine-grained and its volume decreases.

Table 3

The study of the foaming ability of the base of disodium laureth sulfosuccinate, 35% solution of cocamidopropyl betaine and coco glucoside / glyceryl oleate

The concentration of coco glucoside / glyceryl oleate, %	Foaming ability	
	Foam number, mm	Foam stability, c.u.
1.0	123.0	0.94
2.0	114.0	0.95
3.0	106.0	0.96
4.0	92.0	0.97
5.0	66.0	0.97

Table 4

The study of the foaming ability of the base of disodium laureth sulfosuccinate, 35% solution of cocamidopropyl betaine and glyceryl cocoate

The concentration of glyceryl cocoate, %	Foaming ability	
	Foam number, mm	Foam stability, c.u.
0.5	128.0	0.95
1.0	134.0	0.96
1.5	127.0	0.94
2.0	121.0	0.96

Table 5

The study of the foaming ability of the base of disodium laureth sulfosuccinate, 35% solution of cocamidopropyl betaine, glyceryl cocoate and coco glucoside / glyceryl oleate

Concentration, %		Foaming ability	
glyceryl cocoate	coco glucoside / glyceryl oleate	Foam number, mm	Foam stability, c.u.
0.5	0.5	138.0	0.95
1.0	1.0	142.0	0.96
1.5	1.5	139.0	0.94
2.0	2.0	131.0	0.96

Introduction of an additional foam stabilizer is rational.

Glyceryl cocoate was chosen as another foam stabilizer and another degreasing agent.

It was introduced to the base of disodium laureth sulfosuccinate and cocamidopropyl betaine previously heated on a water bath to the temperature of 40-42°C in the concentrations of 0.5%, 1%, 1.5%, and 2%. The surfactant was completely dissolved with the constant work of the mixer at 40 rev/m for 15 min. As a result, turbid liquid solutions without any odour and colour were

formed. The pH of the solutions obtained was 6.90-6.95; the pH value was adjusted to 5.5 with lactic acid.

As can be seen from the results of the research (Table 4), this surfactant affects the level of the foam in this system, but not enough. The sample with the concentration of 1.0 % glyceryl cocoate has relatively high results (the foam number is 134.0 mm, the foam stability is 0.96 c.u.) compared to other samples.

Thereby, at the next stage both stabilizers were added in the foam cleaning base above selected in the ratio of 1:1.

10% Solution of disodium laureth sulfosuccinate was prepared, then 8% of 35% cocamidopropyl betaine was added. The resulting solution was heated on a water bath to the temperature of 40-42°C. At first coco glucoside / glyceryl oleate was dissolved, carefully stirred for 2-3 min. The component was visually checked for complete dissolution, then the required amount of glyceryl cocoate was added in the resulting solution, mixed thoroughly with a mixer of 40 rev/min for 2-5 min. At this time, the solution was cooled to a temperature below 35°C. The pH value of these solutions was within 6.67-6.85. Lactic acid was added, and pH was adjusted to 5.5. The study was conducted with the required number of the samples previously prepared. The solutions obtained were turbid, almost odourless and colourless.

As can be seen from the results of the research (Table 5), the combination of these foam stabilizers with the optimal concentration of 1% are functionally and economically advantageous for their use in this formulation.

CONCLUSIONS

A foam cleaning base with the complex of modern detergents has been experimentally developed. It has been proven that this base corresponds to physicochemical and consumer properties according to the necessary specifications and is a stable system at the pH value selected (5.5 – 6.5).

REFERENCES

1. Горлов И. // SÖFW J. (русская версия). – 2000. – №1. – С. 44-52.
2. Жук О.В., Баранова І.І. // Збірка тез всеукр. наук.-практ. конф. молодих вчених та студентів за міжнар. участю «Сучасні аспекти медицини і фармації – 2014», 15-16 травня 2014 р., м. Запоріжжя. – 2014. – С. 173-174.
3. Изделия косметические. Метод определения водородного показателя pH: ГОСТ 29188.2-91. – Введ. 01.01.98. – М.: Изд-во стандартов, 1992. – 3 с.
4. Кешишян Е.С. // Медицинский совет. – 2008. – №1-2. – С. 57-60.
5. Марголина А.А., Эрнандес Е.И., Зайкина О.Э. Новая косметология. – М.: ИД «Косметика и медицина», 2002. – 247 с.
6. Поверхностно-активные вещества и композиции / Под ред. М.Ю.Плетнева. – М.: Косметика и медицина, 2004. – 780 с.
7. Рогова Г.Б. // Медицинское обслуживание и организация питания в ДОУ. – 2011. – №6. – С. 35-37.
8. Роїк О.М. Розробка складу та технології детоксикуючого гелю: Дис. ... канд. фармацевт. наук. – Х., 2012. – 151 с.
9. Средства моющие синтетические. Метод определения пенообразующей способности: ГОСТ 22567.1-77 (СТ СЭВ 4155-83). – [Взамен ГОСТ 22567.1-77]. – Введ. 01.05.86. – М.: Изд-во стандартов, 1986. – С. 1-6.
10. Студеникин В.М., Студеникина Н.И. // Лечащий врач. – 2008. – №3. – С. 2-7.
11. Ali S., Yosipovitch G. // Acta Dermatolo-Venereologica. – 2013. – Vol. 93. – P. 261-267.

12. *Handbook of Applied Surface and Colloid Chemistry* / Ed. by K.Holmberg. – New York: John Wiley & Sons Ltd, 2002. – Vol. 1. – 606 p.
13. *Handbook of Cosmetic Science and Technology* / Eds A.O.Barel, M.Paye, H.I.Maibach, Dekker Inc. Marcel. – New York: Basel, 2001. – 902 p.
14. *Multifunctional Cosmetics* / Ed. by R.Shueller, P.Romanowski. – Cambridge: Cambridge University Press, 2003. – 248 p.
15. Schramm L.L. *Surfactants: Fundamentals and Applications in Petroleum Industry* – Cambridge: Cambridge University Press, 2000. – 632 p.
16. Zana R., Xia J. *Gemini Surfactants: Synthesis, Interfacial and Solution-Phase Behavior, and Applications*. – New York: Marcel Dekker, 2004. – 345 p.

РОЗРОБКА СКЛАДУ ШАМПУНЮ ДЛЯ ДІТЕЙ

Л.С.Петровська, О.В.Жук, І.І.Баранова

Ключові слова: шампунь для дітей; детергент; піномиюча основа; піноутворювальна здатність

У дітей, зокрема новонароджених, дуже чутлива і вразлива шкіра. Дитяча косметика підтримує захисні функції шкіри, робить її менш чутливою до різних подразників. Саме тому дітям з перших днів життя потрібна інша, ніж для дорослих, більш безпечна для догляду за шкірою косметика, яка не повинна чинити подразнюючої дії. З метою розробки піномийного засобу (шампуню) для дітей нами був обраний ряд сучасних детергентів аніонного, амфотерного і неіоногенного характеру: динатрію лауреатсульфосукцинат 28% («Euronaat LS 3», Disodium Laureth-3-Sulfosuccinate, «ЕОС», Бельгія), кокамідопропілбетаїн («Cocamidopropyl Betain», «КАО», Японія), кокоглюкозид і гліцерилу олеат («Lamesoft PO 65», Coco Glucoside (and) Glyceryl Oleate, «BASF(ex-Cognis)» Німеччина), ПЕГ-7 гліцерилу кокоат, («Neopal LIS 80», PEG-7 Glyceryl Cocoate, «Industria Chimica Panzeri», Італія). В якості регулятора значення рН використовували молочну кислоту. На основі цих речовин були приготувані піномийні основи у різних концентраціях детергентів. Якість (фізико-хімічні властивості) розроблених піномийних основ оцінювали згідно з прийнятою нормативною документацією України. На першому етапі дослідження встановлено, що використання «Euronaat LS 3» є нераціональним, оскільки він не забезпечує достатнього піноутворення, а значить потребує додавання допоміжних детергентів. На наступному етапі нами з метою покращення фізико-хімічних і споживчих властивостей досліджувалися основи, до яких додавали детергенти з низькою подразнюючою дією: «Cocamidopropyl Betain», «Lamesoft PO 65», «Neopal LIS 80». Було виявлено, що піномийні основи з додатково обраними амфотерними і неіоногенними детергентами покращили властивості розроблюваної піномийної основи: підвищився рівень і стабільність піни, а також її споживчі властивості. Необхідно відзначити, що розроблені основи були стабільними при обраному значенні рН (5,5-6,5).

РАЗРАБОТКА СОСТАВА ШАМПУНЯ ДЛЯ ДЕТЕЙ

Л.С.Петровская, Е.В.Жук, И.И. Баранова

Ключевые слова: шампунь для детей; детергент; пеномоющая основа; пенообразующая способность

У детей, в том числе новорожденных, кожа чувствительная и очень уязвимая. Детская косметика поддерживает защитные функции кожи, делает ее менее восприимчивой к различным раздражителям. Именно поэтому детям с первых дней жизни нужна другая, чем у взрослых, более безопасная по уходу за кожей косметика, которая не должна оказывать раздражающего действия. С целью разработки пеномоющего средства (шампуня) для детей нами был выбран ряд современных детергентов анионного, амфотерного и неионогенного характера: династрия лауретсульфосукцинат 28% («Euronaat LS 3», Disodium Laureth-3-Sulfosuccinate, «ЕОС», Бельгия), кокамидопропилбетаин («Cocamidopropyl Betain», «КАО», Япония), кокоглюкозид и глицерилу олеат («Lamesoft PO 65», Coco Glucoside (and) Glyceryl Oleate, «BASF(ex-Cognis)», Германия), ПЭГ-7 глицерилу кокоат, («Neopal LIS 80», PEG-7 Glyceryl Cocoate, «Industria Chimica Panzeri», Италия). В качестве регулятора pH использовали молочную кислоту. На основе этих веществ были приготовлены пеномоющие основы в различных концентрациях детергентов. Качество (физико-химические свойства) разработанных пеномоющих основ оценивали согласно принятой нормативной документации Украины. На первом этапе исследования установлено, что использование «Euronaat LS 3» является нерациональным, поскольку он не обеспечивает достаточного пенообразования, а значит требует добавления вспомогательных детергентов. На следующем этапе нами с целью улучшения физико-химических и потребительских свойств исследовались основы, к которым добавлялись детергенты с низким раздражающим действием: «Cocamidopropyl Betain», «Lamesoft PO 65», «Neopal LIS 80». Было обнаружено, что пеномоющие основы с дополнительно выбранными амфотерными и неионогенными детергентами улучшили свойства разработанной пеномоющей основы: повысился уровень и стабильность пены, а также ее потребительские свойства. Необходимо отметить, что разработанные основы были стабильны при выбранном значении pH (5,5-6,5).

ОРГАНІЗАЦІЯ ТА ЕКОНОМІКА ФАРМАЦІЇ

Recommended by Doctor of Pharmacy, professor M.M.Slobodyanyuk

UDC 615.371:339.13.021:339.138

ANALYSIS OF THE ASSORTMENT OF IMMUNOBIOLOGICAL MEDICAL PRODUCTS USED FOR CHILDREN ROUTINE IMMUNIZATION AT THE UKRAINIAN PHARMACEUTICAL MARKET

A.A.Kotvitska, O.V.Kononenko

National University of Pharmacy

Key words: immunobiological medical products; routine immunization; pharmaceutical market

Marketing analysis of the assortment structure of immunobiological medical products (IMP) used for children routine immunization has been described in the article. It has been found that the given group of products is represented at the Ukrainian pharmaceutical market by 34 trade names, 65% of which belong to foreign producers, 35% are produced by domestic manufacturers. Among the foreign countries-manufacturers of IMP for children routine immunization Belgium takes the leading place, the share of its registered trade names is 29% of this segment. According to the results of the analysis it has been found that manufacturing a number of vaccines mostly from the foreign "in bulk" form is the feature of domestic IMP production. In particular, such production method for vaccines is used by "PHARMA LIFE" company (Lviv), which forms 33% of the domestic vaccine assortment, and "PHARMEX GROUP" company (Boryspil, Kyiv region), and it is 17% of the assortment. Only "PHARMSTANDARD-BIOLIK" company (Kharkiv) provides the production of vaccines from their own raw materials and forms 50% of the domestic vaccine assortment. During the research vaccines were divided by the amount and direction of action of the components, and mono- and multicomponent vaccines were distributed by the countries-manufacturers. It has been found that mono- and multicomponent vaccines are represented at the Ukrainian pharmaceutical market in an equal amount – 17 trade names. Among the countries-manufacturers of monovaccines the leading place belongs to Ukraine (17.6% of the assortment), among the manufacturers of multicomponent vaccines Belgium takes the first place representing 41% of the assortment. The results of the marketing analysis show the dependence of the Ukrainian market of vaccines, especially multicomponent ones, on import and determine the need for further study of the availability level of IMP for children routine immunization.

The use of vaccines for prevention of infectious diseases proves its efficiency for many years, as evidenced by the nearly 3 million children lives, which according to the World Health Organization can be saved thanks to vaccination every year [20]. According to the experts' opinion the use of new immunobiological medical products (IMP), which will be developed over the next 5-15 years, will enable to prevent mortality of 8 million children per year. The application of modern biotechnologies contributes to continuous improvement of the IMP world market. Today the development of innovative vaccines (DNA-vaccines, vector vaccines, plant vaccines, etc.) and new combined vaccines that can create the immunity against even 7-8 infections is rather active. Scientists also pay attention to the issue of finding new methods for vaccine application (intranasal, dermal). Thus, one may state that the vaccination system serves as a decisive factor in preventing of infant mortality, improving the quality and increasing the life expectancy of for groups of the population [5, 9, 10].

The aim of our work was to analyze the assortment structure of IMP registered in Ukraine for children routine immunization with determination of the current trends and problems in this segment of the domestic pharmaceutical market.

Materials and Methods

During the research we used the information from the State Register of medicines of Ukraine as of 08.01.2014 and the reference book "Compendium – Medicines".

Mathematical and graphical methods of analysis were used for the study.

Results and Discussion

According to the Order of the Ministry of Public Health of Ukraine from 16.09.2011 No.595 "About the procedure of prophylactic immunization in Ukraine and control of the quality and turnover of immunobiological medical products" the compulsory children immunization by age is provided against 10 infectious diseases, namely *diphtheria, pertussis, measles, poliomyelitis, tetanus, tuberculosis, rubella, mumps, hepatitis B and Haemophilus influenza b* [6].

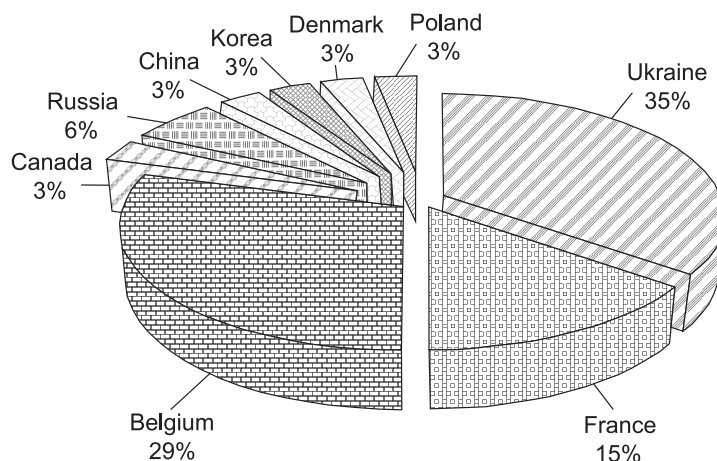


Fig. 1. The ratio of IMP registered in Ukraine for routine immunization of children according to the countries-producers.

According to the ATC classification system the IMP used for routine immunization of children belong to group J – Antimicrobial medications for systemic use and constitute a subgroup of the second level J07 – Vaccines.

Based on data from the State Register of medicines of Ukraine it was found that 34 IMP trade names that could be used for routine vaccination of children were registered in Ukraine on 08.01.2014 [1, 2].

The analysis of the IMP assortment according to the countries-producers has shown that 65% (22 trade names) of the specified segment belong to foreign producers and only 35% (12 trade names) – to domestic ones. Results of the analysis of the assortment structure of foreign IMP suggest that the Belgian vaccines take the leading position at the market, namely 29% (10 trade names). The French manufacturer offers 5 vaccines to the market, and it is 15% of the segment. The market share of IMP consisting of the Russian companies is equal to 6% (2 trade names). The smallest shares, namely 3% of the market each, are occupied by such countries as Canada, China, Korea, Denmark and Poland; they produce 1 vaccine for the domestic market (Fig. 1) [4].

The characteristic feature of domestic production of IMP is that a quite significant share of vaccines is produced mainly from foreign “in bulk” forms. Bulk drugs are pharmaceutical products intended for manufacture of finished products and passed all stages of the production process, except for the final packaging and labeling by another manufacturer. The essence of the production process is that a manufacturing company makes a product and sends it to another company that is engaged in its packaging and labeling.

Therefore, the next stage of our research was sorting of domestic drugs registered in Ukraine according to the manufacturers. Based on the results of the research it has been found that only “Pharmstandard-Biolik” JSC (Kharkiv) provides the production of vaccines from their own raw materials; it has 6 vaccines at the market, and it is 50% of the domestic IMP registered for routine children immunization. These vaccines include diphtheria toxoid (*Dip-M-Biolik*), tetanus toxoid (*TT-Biolik*), tetanus toxoid in combination with diphtheria toxoid (*DT-*

Biolik, *DT-M-Biolik*), acellular combined adsorbed vaccine for prevention of diphtheria, tetanus and pertussis (*DTP-Biolik*) and *recombinant liquid vaccine for prevention of hepatitis B*. “PHARMA LIFE” company (Lviv) provides 33% of the domestic vaccine assortment and is represented by 4 trade names. In particular, from “in bulk” form of GlaxoSmithKline Biologicals s.a. company (Belgium) this company produces vaccine against hemophilus influenzae b *HIBERYKS*, vaccine against diphtheria, tetanus and pertussis *INFANRIX*, vaccine against measles, mumps and rubella *PRIORIX* and from “in bulk” form of the Institute of Immunology Inc. (Croatia) – vaccine against rubella *KRASLAYF*. “FARMEKS GROUP” company (Boryspil, Kyiv region) produces 2 vaccines (17% of the assortment) – vaccine against poliomyelitis *IMOVAKS POLIO* and vaccine against diphtheria, hemophilus influenzae b, pertussis, poliomyelitis and tetanus *PENTAXIM* (both – from the “in bulk” form of the company Sanofi Pasteur, France) (Fig. 2).

In present-day conditions new vaccines that can simultaneously create protection against several infectious diseases are continuously developed [15, 19]. It should be mentioned that vaccines, which after their introduction create the immunity against several infectious diseases, are called multicomponent (polyvalent) vaccines, and vaccines, which provide the possibility of creating

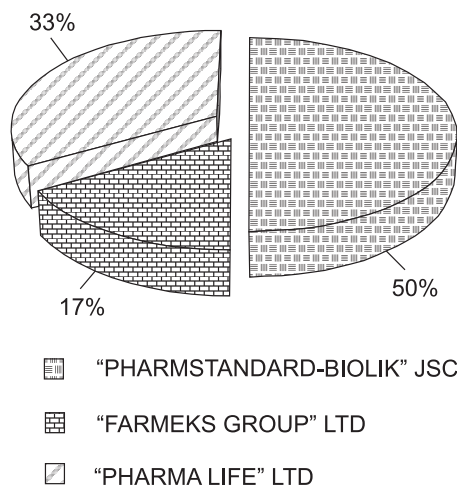


Fig. 2. The ratio of domestic IMP for routine immunization of children according to the manufacturers.

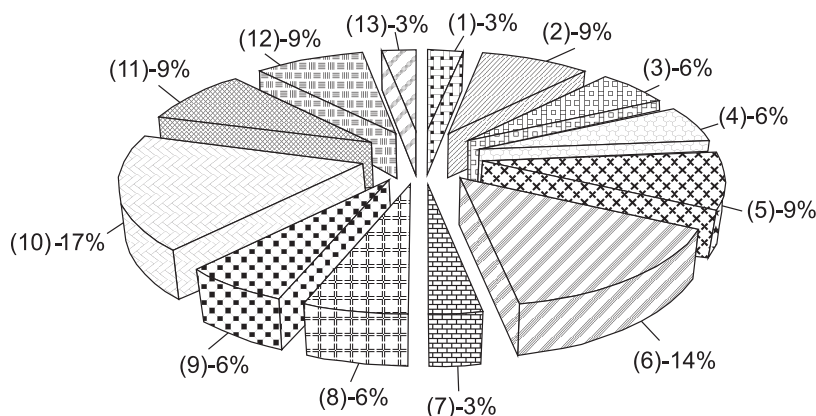


Fig. 3. The ratio of IMP for the routine immunization of children by the amount and direction of action of the components:
 1 – Vaccine against diphtheria; 2 – Vaccine against hemophilus influenzae b; 3 – Vaccine against tetanus; 4 – Vaccine against tuberculosis;
 5 – Vaccine against hepatitis B; 6 – Vaccine against poliomyelitis; 7 – Vaccine against rubella; 8 – Vaccine against diphtheria and tetanus;
 9 – Vaccine against measles, mumps and rubella; 10 – Vaccine against pertussis, diphtheria and tetanus; 11 – Vaccine against diphtheria, pertussis, poliomyelitis and tetanus; 12 – Vaccine against diphtheria, hemophilus influenzae b, pertussis, poliomyelitis and tetanus;
 13 – Vaccine against diphtheria, hemophilus influenzae b, pertussis, poliomyelitis, tetanus and hepatitis B.

the immunity against only one infection, are called monocomponent. The advantages of the multicomponent vaccines are [8]:

- decrease in the content of adjuvants, preservatives and stabilizers while using a combined vaccine comparing to application of several monovaccines; it reduces the risk of immunization reactions and complications [13];
- cost savings (the cost of one vaccine in the composition of a combined vaccine is less than the cost of a monovaccine);
- reduction of the number of visits to a doctor, saving of the parents' time;
- decrease of the emotional stress of the child;
- more timely and complete fulfillment of the prophylactic immunization chart [17];
- reduction of the number of injections during the vaccination according to the National vaccination calendar. Thus, the use of combined vaccines allows to immunize a child in the first 18 months of life with 9-11 vaccinations while using monovaccines involves 14-17 vaccinations [7, 12, 18].

In view of the abovementioned, the next step of our study was the classification of vaccines used for routine immunization of children according to the amount and direction of action of the components. Based on the results of the research it has been found that the combined vaccine against *diphtheria, tetanus and pertussis* (DTP), which is represented by 6 trade names, takes the largest share at the market (Fig. 3). The vaccine mentioned is one of the first polyvalent vaccines created and used in Ukraine in the course of preventive vaccination over 60 years. Other combined vaccines that include these three components were created exactly on the basis of DTP-vaccine. Somewhat smaller share in the market, namely 14% (5 trade names), is taken by vaccine against *polio*. Monovaccines against *hemophilus influenzae b* and *hepatitis B*, as well as combined vaccines against *diphtheria, pertussis, polio and tetanus* and against *diphtheria, hemophilus influenzae b, pertussis, polio and tetanus* occupy 9% of the studied assortment, and it is 3

trade names each. The vaccine against *tuberculosis* and the vaccine against *tetanus* are 6% (2 trade names) of the segment analyzed. The similar situation is observed with polyvalent vaccines against *diphtheria and tetanus* and against *measles, mumps and rubella*. Monovalent vaccines against *diphtheria* and against *rubella* and a six-component vaccine against *diphtheria, hemophilus influenzae b, pertussis, polio, tetanus and hepatitis B* take 3% of the segment of the market under research represented only by 1 trade name each [3].

The production of **multicomponent vaccines** indicates a considerable state interest in organizing and conducting vaccination, as well as its willingness to spend significant resources in this healthcare branch due to the fact that the production of vaccines is much less lucrative than, for example, of antibiotics [11, 14, 16]. Therefore, the next stage of our study was the structural analysis of countries-manufacturers of monovaccines and multicomponent vaccines registered in Ukraine, the results of which are given in Table, Fig. 4 and 5.

According to the research results **monovaccines** and **combined vaccines** are represented at the domestic pharmaceutical market in equal amounts – 17 trade names. Among the countries-manufacturers of monovaccines the leading place belongs to Ukraine, which produces 6 trade names (17.6% of the assortment) for the market, however, 3 of these 6 vaccines are made not of the own raw material, but from “in bulk” forms of foreign manufacturers. Shares that make up 8.8% are occupied by manufacturers from France and Belgium. Vaccines produced in Russia are 5.9% of the assortment analyzed, and the least amount of the vaccines registered in Ukraine (2.9% of the assortment) belong to Denmark, Poland and Korea.

Two-component vaccines are produced for the market only by the Ukrainian manufacturers and are up to 5.9% of the IMP registered for the routine immunization of children.

Among the countries-manufacturers of **three-component vaccines** by the number of the registered IMP the leading places belong to Belgium and Ukraine, each of them offer 3 vaccines of the given composition (8.8%,

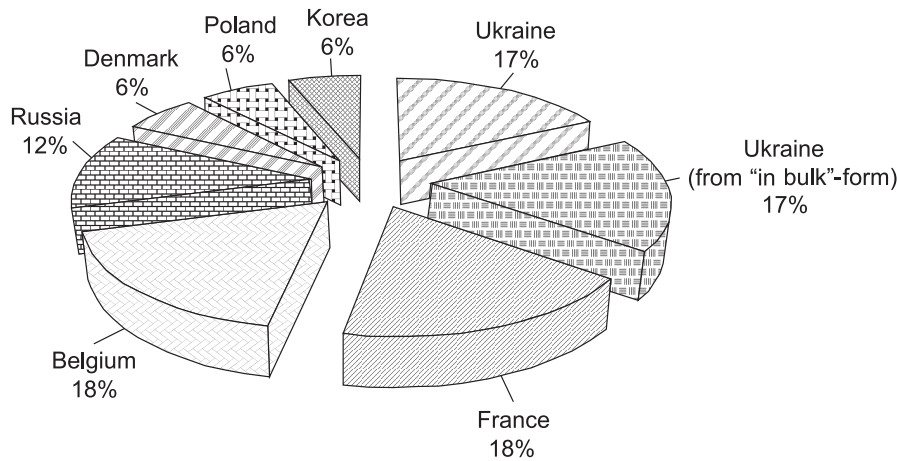


Fig. 4. The structure of countries-manufacturers of *monovaccines* according to the State Register of medicines of Ukraine.

Table

The ratio of monovaccines and multicomponent vaccines registered in Ukraine according to countries-manufacturers

Country-manufacturer	The number of the trade names registered	The share, %
1	2	3
Monovaccines		
Ukraine	3	8.8
Ukraine (from foreign "in bulk" form)	3	8.8
France	3	8.8
Belgium	3	8.8
Russia	2	5.9
Denmark	1	2.9
Poland	1	2.9
Korea	1	2.9
In all:	17	50
Two-component vaccines		
Ukraine	2	5.9
Three-component vaccines		
Canada	1	2.9

Table continued

1	2	3
Belgium	3	8.8
China	1	2.9
Ukraine (from foreign "in bulk" form)	2	5.9
Ukraine	1	2.9
In all:	8	23.5
Four-component vaccines		
Belgium	2	5.9
France	1	2.9
In all:	3	8.8
Five-component vaccines		
Belgium	1	2.9
France	1	2.9
Ukraine (from foreign "in bulk" form)	1	2.9
In all:	3	8.8
Six-component vaccine		
Belgium	1	2.9
The total number:	34	100

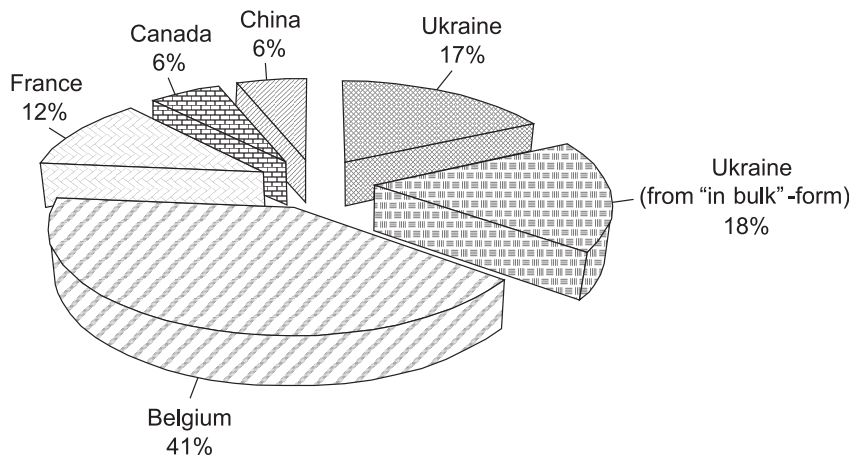


Fig. 5. The structure of countries-manufacturers of *multicomponent vaccines* according to the State Register of medicines of Ukraine.

respectively). However, 2 of 3 of the Ukrainian registered vaccines are produced from foreign "in bulk" form and only one – from the own raw material.

Four-component vaccines are offered to the market only by foreign manufacturers, 2 of them belong to the Belgian manufacturer and 1 – to the French manufacturer, it is 5.9% and 2.9%, respectively.

Countries-manufacturers of **five-component vaccines** occupy a share of 8.8% of the current assortment uniformly distributed between France, Belgium and Ukraine, each of them offer 1 vaccine to the market. It should be noted that the Ukrainian companies manufacture vaccines from foreign "in bulk" forms.

Only Belgium produces a **six-component vaccine** to the Ukrainian pharmaceutical market, its share is equal to 2.9% of the assortment.

Thus, according to the results of the studies there is a clear tendency of dependence of the IMP domestic market on the import of both manufactured vaccines and the raw material for their manufacture by domestic companies.

CONCLUSIONS

1. It has been found that in the assortment of IMP for the routine immunization of children the foreign vaccines are dominated, their share is 65% (22 trade names). Domestic manufacturers offer 35% of the assortment at the market (12 trade names).

2. Among domestic manufacturers by the number of the vaccines registered in Ukraine "Pharmstandard-Biolik" JSC (Kharkiv) takes the leading place, it produces 6 vaccines for the market, and it is 50% of the domestic registered IMP for routine immunization of children.

3. Monovaccines and multicomponent vaccines are represented in an equal amount at the pharmaceutical market. The leader in production of monovaccines is Ukraine, of multicomponent vaccines – Belgium.

Taking into account a rather difficult situation for the population attitude towards vaccination in Ukraine our future research will be aimed at determining the availability of information about IMP for routine immunization of children.

REFERENCES

1. Державний реєстр лікарських засобів України [Електронний ресурс]. – Режим доступу: <http://www.drlez.kiev.ua/>.
2. Компендиум on-line. [Електронний ресурс]. – Режим доступу: <http://compendium.com.ua/>.
3. Котвицкая А.А., Кононенко О.В. // Матер. 4-ой Междунар. науч.-практ. конф. студентов и молодых ученых «Фармацевтический кластер как интеграция науки, образования и производства». – Белгород.: ИД «Белгород» НИУ БелГУ, 2013. – С. 13-15.
4. Котвицька А.А., Кононенко О.В. // Матер. II Міжнар. наук.-практ. Internet-конф. «Менеджмент та маркетинг у складі сучасної економіки, науки, освіти, практики». – Х.: Вид-во НФаУ, 2014. – С. 245-246.
5. Марієвський В.Ф. // Сучасні інфекції. – 2009. – №3-4. – С. 7-11.
6. Наказ МОЗ України від 16.09.2011 №595 «Про порядок проведення профілактичних щеплень в Україні та контроль якості і обігу медичних імунобіологічних препаратів» [Електронний ресурс]. – Режим доступу: <http://zakon3.rada.gov.ua/laws/show/z1159-11>.
7. Платов С.М., Заглада О.О., Мендрік О.А. // Клин. иммунол. Аллергол. Инфектол. – 2011. – №2. – С. 11-15.
8. Устінов О.В., Чудутова Д. // Укр. мед. часопис. – 2010. – №5(79). – С. 33-36.
9. Advani A. Epidemiological characterisation of Bordetella pertussis in Sweden, 1970-2004. – Stockholm, 2007. – P. 1-59.
10. Edelman K., He Q., Mäkinen J. et al. // Clin. Infect. Dis. – 2007. – Vol. 44. – P. 1271-1277.
11. Keith J.A., Bigger L.A., Arthur P.A. et al. // Vaccine. – 2013. – Vol. 31. – P. 184-193.
12. Mast E.E., Ward J.W. Vaccines. 5th ed. – China, 2008. – P. 205-241.
13. Offit P.A., Quarles J., Gerber M.A. et al. // Pediatrics. – 2003. – Vol. 109. – P. 124-29.
14. Orenstein W.A., Rodewald L.E., Hinman A.R. et al. Vaccines. 5th ed. – China, 2008. – P. 1479-1510.
15. Plotkin S., Stanley A., Orenstein W. et al. Vaccines. 5th ed. – China, 2008. – 1748 P.
16. Plotkin S.L., Plotkin S.A. Vaccines. 5th ed. – China, 2008. – P. 345-357.
17. Recommendations of the Advisory Committee on Immunization Practices (ACIP), the American Academy of Pediatrics (AAP), and the American Academy of Family Physicians (AAFP). – 2005. – Vol. 48. [Електронний ресурс]. – Режим доступу: <http://www.cdc.gov/mmwr/preview/mmwrhtml/rr4805a1.htm>.
18. Swedish Institute for Infectious Disease Control. – Ten year Report: October 1, 1997 until December 31, 2007. – P. 1-53.
19. Taylor K., Nguyen A., Stéphenne J. // Vaccine. – 2009. – Vol. 27. – P. 3-8.
20. WHO, UNICEF, World Bank. State of the world's vaccines and immunization. 3rd ed. – Geneva, World Health Organization, 2009 [Електронний ресурс]. – Режим доступу: http://whqlibdoc.who.int/publications/2009/9789241563864_eng.pdf.

АНАЛІЗ АСОРТИМЕНТУ МЕДИЧНИХ ІМУНОБІОЛОГІЧНИХ ПРЕПАРАТІВ, ЩО ВИКОРИСТОВУЮТЬСЯ ДЛЯ ПЛАНОВОЇ ВАКЦИНОПРОФІЛАКТИКИ ДІТЕЙ НА ФАРМАЦЕВТИЧНОМУ РИНКУ УКРАЇНИ**А.А.Котвицька, О.В.Кононенко****Ключові слова:** медичні імунобіологічні препарати; планова вакцинопрофілактика; фармацевтичний ринок

Проведено маркетинговий аналіз асортиментної структури медичних імунобіологічних препаратів (МІБП), які використовуються для планової вакцинопрофілактики дітей. Встановлено, що зазначена група препаратів представлена на українському фармацевтичному ринку 34 торговими назвами, з яких 65% належать іноземним виробникам, а 35% виробляються вітчизняними виробниками. Серед іноземних країн-виробників МІБП для планової вакцинопрофілактики дітей лідируюче місце посідає Бельгія, частка зареєстрованих торгових назв якої становить 29% від зазначеного сегменту. За результатами досліджень встановлено, що характерною особливістю вітчизняного виробництва МІБП є виготовлення низки вакцин переважно з форми *in bulk* іноземного виробництва. Зокрема такий спосіб виробництва вакцин використовується компаніями ТОВ «ФАРМА ЛАЙФ» (м. Львів), якою формується 33% асортименту вітчизняних вакцин, та ТОВ «ФАРМЕКС ГРУП» (м. Бориспіль, Київська обл.), що складає 17% асортименту. Виготовлення вакцин з власної сировини здійснює лише ПАТ «ФАРМСТАНДАРТ-БІОЛІК» (м. Харків), що формує 50% асортименту вітчизняних вакцин. У ході досліджень проведено розподіл вакцин за кількістю та напрямом дії компонентів, а також розподіл моно- та багатокомпонентних вакцин за країнами-виробниками. Встановлено, що моно- та багатокомпонентні вакцини представлені на вітчизняному фармацевтичному ринку в рівній кількості – по 17 торгових назв. Серед країн-виробників моновакцин лідируюче місце належить Україні (17,6% асортименту), серед виробників багатокомпонентних вакцин перше місце посідає Бельгія, що складає 41% асортименту. Результати проведеного маркетингового аналізу свідчать про залежність українського ринку вакцин, особливо багатокомпонентних, від імпорту, що зумовлює необхідність подальшого вивчення рівня забезпеченості населення МІБП для планової вакцинопрофілактики дітей.

АНАЛИЗ АССОРТИМЕНТА МЕДИЦИНСКИХ ИММУНОБИОЛОГИЧЕСКИХ ПРЕПАРАТОВ, КОТОРЫЕ ИСПОЛЬЗУЮТСЯ ДЛЯ ПЛАНОВОЙ ВАКЦИНОПРОФИЛАКТИКИ ДЕТЕЙ НА ФАРМАЦЕВТИЧЕСКОМ РЫНКЕ УКРАИНЫ**А.А.Котвицкая, О.В.Кононенко****Ключевые слова:** медицинские иммунобиологические препараты; плановая вакцинопрофилактика; фармацевтический рынок

Проведен маркетинговый анализ ассортимента структуры медицинских иммунобиологических препаратов (МИБП), используемых для плановой вакцинопрофилактики детей. Установлено, что указанная группа препаратов представлена на украинском фармацевтическом рынке 34 торговыми названиями, из которых 65% принадлежат иностранным производителям, а 35% производятся отечественными производителями. Среди иностранных стран-производителей МИБП для плановой вакцинопрофилактики детей лидирующее место занимает Бельгия, часть зарегистрированных торговых названий которой составляет 29% от указанного сегмента. По результатам исследований установлено, что характерной особенностью отечественного производства МИБП является изготовление ряда вакцин преимущественно из формы *in bulk* иностранного производства. В частности такой способ производства вакцин используется компаниями ООО «ФАРМА ЛАЙФ» (г. Львов), которые формируют 33% ассортимента отечественных вакцин, и ООО «ФАРМЕКС ГРУПП» (г. Борисполь, Киевская обл.), что составляет 17% ассортимента. Изготовление вакцин из собственного сырья осуществляет только ОАО «ФАРМСТАНДАРТ-БИОЛЕК» (г. Харьков), который формирует 50% ассортимента отечественных вакцин. В ходе исследований проведено распределение вакцин по количеству и направлению действия компонентов, а также распределение моно- и многокомпонентных вакцин по странам-производителям. Установлено, что моно- и многокомпонентные вакцины представлены на отечественном фармацевтическом рынке в равном количестве – по 17 торговых названий. Среди стран-производителей моновакцин лидирующее место принадлежит Украине (17,6% ассортимента), среди производителей многокомпонентных вакцин первое место занимает Бельгия, что составляет 41% ассортимента. Результаты проведенного маркетингового анализа свидетельствуют о зависимости украинского рынка вакцин, особенно многокомпонентных, от импорта, что обуславливает необходимость дальнейшего изучения уровня обеспеченности населения МИБП для плановой вакцинопрофилактики детей.

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

UDC 615.15:349.3

ANALYSIS OF THE ATTITUDE OF PHARMACY SPECIALISTS TOWARDS THE CURRENT SOCIAL PROTECTION SYSTEM AND DIRECTIONS FOR ITS REFORMATION

M.V.Zarichkova

National University of Pharmacy

Key words: social protection of pharmacy specialists; social services; pharmaceutical industry; authorised person responsible for social issues; labour protection; ADPQ_(SPPhS) software application

The social status of pharmacy specialists (PhS) of the wholesale and retail branch of the pharmaceutical industry of Ukraine and their attitude towards the current social protection (SP) system have been analysed. The possibilities of PhS to gain social protection at their workplace, participate in social programmes and offers to reform the current SP system have been studied. It has been found that civil and self-regulating organisations in the system of social protection of pharmacy specialists (SPPhS) are of primary importance. This will enable to improve relations between participants of SPPhS and affect efficiently the solution of disputes between a PhS and an employer. For this purpose it has been offered to assign an authorised person who is responsible for social issues in the labour collective of pharmaceutical institutions of all types of ownership. The software application ADPQ_(SPPhS) (Automatic data processing questionnaires (the attitude to the current social protection for pharmacy specialists (SPPhS))) has been developed for the first time with the purpose of processing the results of SPPhS research of the wholesale and retail branch of the pharmaceutical industry of Ukraine.

The pharmaceutical industry is important and it is one of the strategic sectors of the economy of Ukraine because it is responsible for health of the nation and provision of the population with medicines and medical supplies. Taking into account the fact that work of pharmacy specialists (PhS) is associated with emotional tension during all working hours (a human factor), and that they deal with different categories of medicines (narcotic, toxic, potent, radioactive, the plant medicinal raw material, etc.), which can cause a threat to health (occupational diseases), employees of the pharmaceutical industry need special protection by the state and employers, especially it concerns social protection (SP). Today social protection of pharmacy specialists (SPPhS) is implemented in the system of SP of the population, but unfortunately, there is no separate state regulation of SPPhS. This caused conducting a sociological study of PhS of the wholesale and retail branch of the pharmaceutical industry of Ukraine to determine their social status and attitude towards the current system of SP [1-4, 9, 10].

Our study was conducted in two phases; thereby contributed to obtaining more detailed information from PhS – potential consumers of social services at wholesale and retail enterprises of the pharmaceutical industry of Ukraine.

The first phase was devoted to determination of the social status of the PhS interviewed and their positioning in the SP system of Ukraine. The respondents were PhS of the wholesale and retail branch of the pharmaceutical industry of Ukraine.

The second phase was directed to identification of the attitude of the PhS interviewed towards the current

SP system, their ability to be assisted at their workplace, participation in social programmes and identification of the offers concerning the reformation of the current system of SPPhS.

Materials and Methods

The study was conducted based on the social survey using questionnaires for PhS of the wholesale and retail branch of the pharmaceutical industry of Ukraine in Kyiv, Dnipropetrovsk, Donetsk, Luhansk, Poltava, Sumy and Kharkiv regions. The total amount of the study sample constituted 1000 respondents. The software application ADPQ_(SPPhS) (Automatic data processing questionnaires (the attitude to the current social protection for pharmacy specialists (SPPhS))) was used for the first time to process the results obtained. It has been developed at the Department of Management and Economics of Pharmacy of the Institute of Pharmacy Professionals Qualification Improvement at the National University of Pharmacy. This software processes and analyses the data obtained, allowing access from specific facts to general conclusions and, in some cases, making a forecast of the study process development [5-9, 11].

Results and Discussion

Social and demographic characteristic of the PhS participated in the study. We interviewed PhS, who occupy different posts: Head of a pharmacy; Deputy Head of a pharmacy; Head of a Department; Deputy Head of a Department; Head of a warehouse; Head of a pharmacy outlet; Director; Senior Pharmacist; Pharmacist; Office Worker.

The PhS participated in our study work at wholesale and retail pharmacies of different forms of ownership:

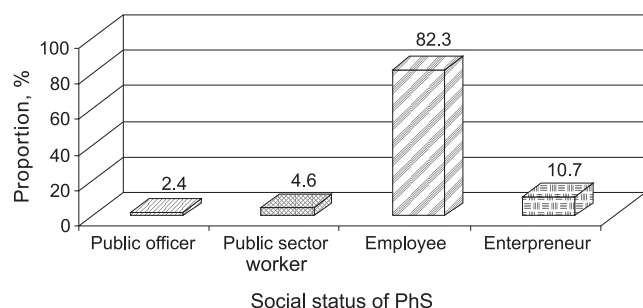


Fig. 1. Division of PhS according to their social status.

state, municipal and private. In particular, they are the staff of such pharmaceutical institutions as pharmacy, pharmacy outlet, pharmacy warehouse, office of a pharmaceutical company.

According to their social status PhS were divided into four categories shown in the graph (Fig. 1).

As shown in Fig. 1, 2.4% of PhS are public officers, 4.6% of PhS are public sector workers, 82.3% of PhS are employees and 10.7% of PhS are entrepreneurs. Most PhS are employees who depend on the company where they work financially and socially; and according to our study, not all of them feel socially protected.

Among the respondents, most PhS use SP in the SP system of the population and they have not experienced the participation in SPPHS, they use mostly SP programmes shown in Fig. 2.

As shown in Fig. 2, according to the percentage most PhS of our social survey participate in such state programmes of social protection as: a labour veteran – 6.1%; a disabled person of group I; a disabled person of group II; a disabled person of group III – 2.1%; a war veteran; a child of war; a combatant; a disabled person of the Great Patriotic War – 1.5%.

According to our data, the priority of public social programmes is based on rather important parameters, which characterize their quality and credibility level to the aimed social impact. With regard to other existing social programmes, it has been found that the PhS interviewed are not experienced in the use of them and are unable to obtain the desired SP by the employer [10].

Our studies have shown that PhS – entrepreneurs find ways of protection by themselves and they do not wait for any assistance from the state and employers, so that they do not use the existing state programmes of SP.

Generally, only 13% of the PhS interviewed have applied for social support at workplace and received some of it. Other 87% have not had such experience, chance and desire, and they have not got social support at the workplace. The abovementioned fact indicates the absence of effective cooperation between an employer and a PhS concerning social stability and prosperity. This may lead to conflicts between a PhS and an employer, and as a result, may lead to conflicts between them and the executive authorities.

Complexity and variety of unsolved social problems, expansion of the range of demands in social services among PhS result in the need to implement a lot of pressing problems, namely to search scientific reasonable options and models of SPPHS provision, to determine the place and part of social security in it, to develop forms, methods and means of protection against threats and emergencies of the social nature, as well as to train the qualified personnel, who are competent to solve the abovementioned problems.

We recommend assigning an authorized person who is responsible for social issues (APSI) at the labour collective of pharmaceutical institutions of all types of ownership as one of the ways to settle the issues mentioned. We also suggest to determine qualifying requirements towards the APSI's activity and to develop duty regulations. It is also advisable to enhance the role of civil and self-regulated organisations in the SPPHS system. Assigning an APSI will enhance the role of the labour collective in the SPPHS system and will make the SP system more effective and focused on the current needs for social services of PhS in Ukraine [2, 3, 4].

It has been found that most of the PhS interviewed (52.3%) do not participate in the programmes of the social security insurance and obtaining of social benefits (Fig. 3).

According to the ranking conducted, retirement pensioners are at the top – 15.5%. According to the degree of demand, there are social insurance programmes, in-

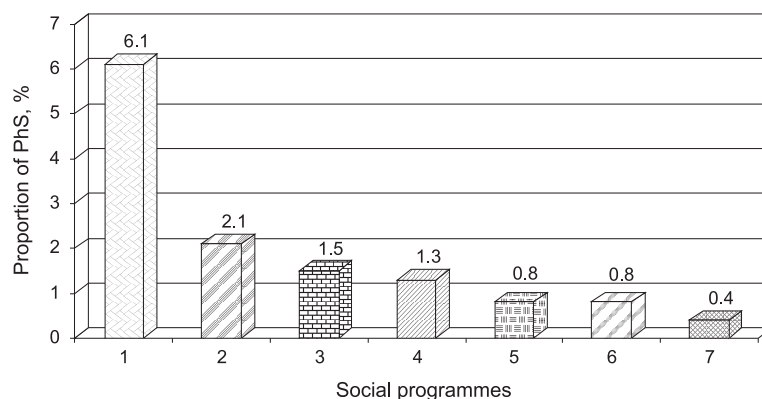


Fig. 2. Social protection programmes, in which the PhS under study participate.
 Symbols: 1 – labour veteran; 2 – disabled person of group I; disabled person of group II; disabled person of group III; 3 – war veteran; child of war; combatant; disabled person of the Great Patriotic War; 4 – liquidators of the Chernobyl disaster; person affected by the Chernobyl disaster; 5 – single mother; 6 – other categories; 7 – large family.

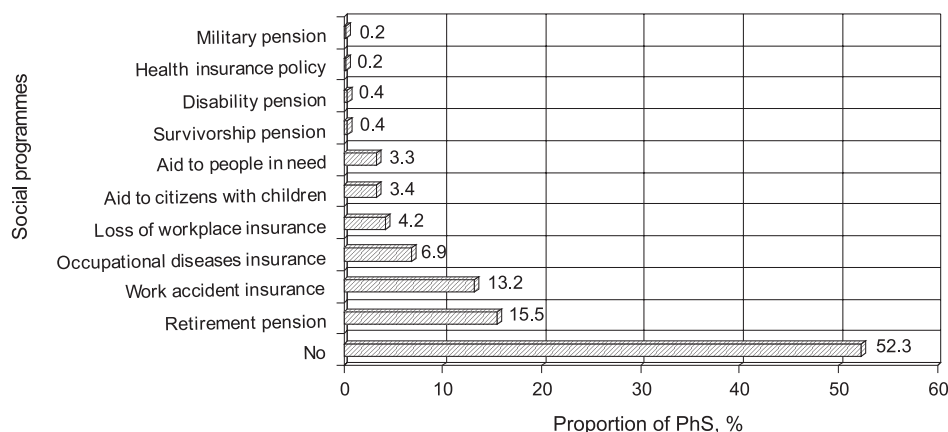


Fig. 3. Social benefits received by the PhS interviewed.

cluding the work accident insurance – 13.2%, the occupational diseases insurance – 6.9%, the loss of workplace insurance – 4.2%. Finally, the lowest percentage accounts for PhS participating in such social programmes as aid to citizens with children – 3.4%; aid to people in need – 3.3%; survivorship pension – 0.4%; disability pension – 0.4%; military pension – 0.2%; health insurance policy – 0.2%.

During the second phase we have found out the attitude of PhS towards the SP system at workplaces and the opportunities to use SP. It is known that there are many structures that must provide the population with SP in Ukraine. However, the legislation does not provide certain SP for PhS taking into account the specificity of their activities. Therefore, reformation of the SP system of Ukraine and separation of SPPHS into an independent branch within the general system of SP of the population require establishment of the executive body. For these reasons the credibility level of PhS and effectiveness of SPPHS providing among the existing institutions concerning SP have been found during the survey. The study results are presented in Fig. 4.

As shown in Fig. 4, the majority of the PhS interviewed – 45% express confidence in the Ministry of Labour and Social Policy (in cooperation with the Employment Fund). This authority is responsible for the current state of SPPHS within the system SP of the population. 25% of the PhS interviewed would like an employer to

be responsible for SPPHS. According to the opinion of 19% of the respondents, the sector trade unions should be responsible for PhS and provide them with SP. Private insurance funds receive 5% of the PhS' confidence, and it is clear taking into account the current state of the economy of Ukraine.

Credibility of the SPPHS performance by the councils of labour groups is expressed only by 3% of the respondents. It is explained by the lack of experience of such work among labour collectives in the pharmaceutical industry.

Based on these facts we can conclude that there is a need to enhance the role of civil and self-regulated organisations in the SPPHS system. It will make relations between the parties of SPPHS efficient, and solve all issues on SP between a PhS, an employer and the executive authority in the optimal way.

During the study we simulated the following situation in the experiment: respondents were asked to describe the components of the concept "social protection of pharmacy specialists". It has been found that the system of SP of the population should include such concept as "social protection of pharmacy specialists". It is also necessary to develop the system of social services and SPPHS provision by employers within the pharmaceutical industry of Ukraine. In general, it has been determined that a large number of concepts listed in Fig. 5 is important for PhS while defining the SPPHS [4].

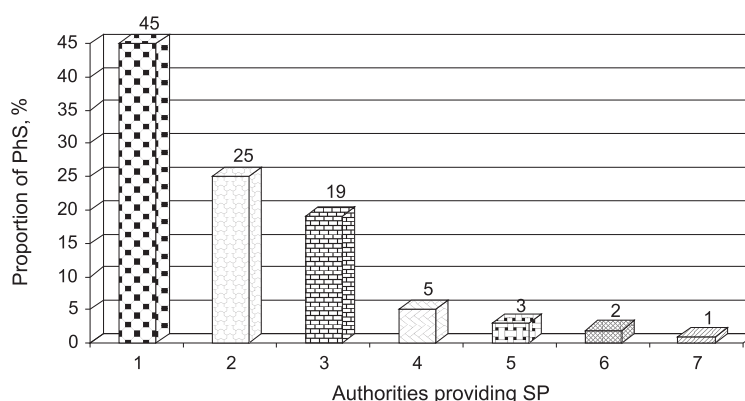


Fig. 4. Credibility in the authorities providing SPPHS.

Symbols: 1 – the Ministry of Labour and Social Policy in cooperation with the Employment Fund; 2 – employer; 3 – sector trade unions; 4 – private insurance funds; 5 – the council of the labour collective; 6 – a new structure; 7 – Verkhovna Rada (Parliament), the Ministry of Public Health.

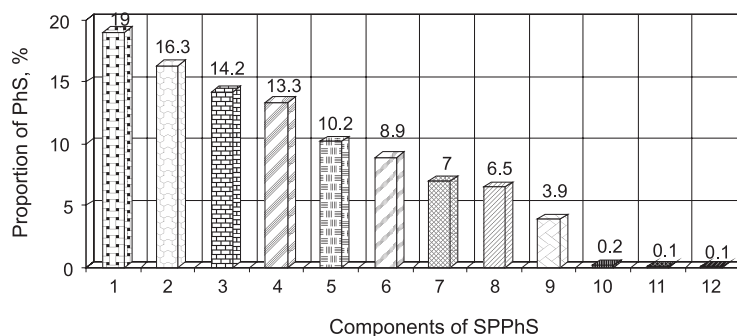


Fig. 5. The PhS attitude towards the components of the definition of SPPhS.

Symbols: 1 – social insurance; 2 – income provision in case of disability or loss of workplace; 3 – treatment and prevention of occupational diseases; 4 – protection of social rights and minimum guarantees; 5 – assistance in education and advanced training of pharmacy specialists; 6 – social support and assistance; 7 – protection against professional burnout; 8 – social support of disabled citizens; 9 – healthy lifestyle promotion; 10 – wage; 11 – improvement in health and spa facilities; 12 – behavioural orientation “do not leave people to the mercy of fate”.

As shown in Fig. 5, the majority of the PhS interviewed (19%) would like to have social security insurance for their protection. Concern for their status when losing the workplace is the following, so 16.3% of the PhS interviewed want to be insured and their income is secured in case of disability or loss of workplace. This concern is caused by economic instability in Ukraine and by fear to be unemployed and to lose initial assets.

The need in treatment and prevention of occupational diseases takes the third place among the PhS interviewed (14.2%). There are almost no measures on labour protection (LP) at workplaces today. The importance of preventive measures on LP is widely recognized in various sectors of the economy; and as for the pharmaceutical industry, this issue is considered in the manufacturing sector mainly, but a lot of pressing issues on this case are still unresolved in pharmacies and their structural subdivisions.

At the same time LP is one of the responsibilities of the state and the society. Not only specialists that are responsible for LP issues at the enterprise level, but also numerous ordinary and new participants are involved into LP. The health of PhS and performance indicators are based not only on production factors, but also on non-production ones. Therefore, this problem-solving approach should be more integrated and holistic with involvement of various parties who have different experience and skills. At the pharmacy level one of the main obstacles to improve LP is the opinion that the expenses for the prevention of LP are unprofitable and they decrease the competitiveness of a pharmacy [2, 3].

According to the opinion of the PhS interviewed the following components are also important: 13.3% – protection of social rights and minimum guarantees; 10.2% – assistance in education and advanced training of pharmacy specialists; 8.9% – social support and assistance; 7% – protection against professional burnout; 6.5% – social support of disabled citizens, etc.

The studies conducted confirm the need to reform the current SP system and to separate SPPhS into an independent branch.

CONCLUSIONS

1. The social status of PhS and their attitude towards the current SP system at the workplace have been studied. It has been found out that the current legislation regulating the implementation of SP, should be reconsidered, improved and harmonized with the EU policy documents. Taking into account the specificity of the pharmaceutical industry it is necessary to create an independent system of SPPhS. This requires updating of the SPPhS legislative framework, which must be based on the European principles and take into account the needs of PhS and employers.

2. We have created a ADPQ_(SPPhS) computer programme. It is efficient when conducting sociological surveys with a great number of respondents and questions. It allows taking into account most indicators of the qualitative nature as there is a human factor when questioning, so it enables obtaining more reliable results of the statistical data processing. It allows interpreting the data obtained scientifically and using them in the further studies.

3. There is a need to enhance the role of civil and self-regulated organisations in the SPPhS system nowadays. It will make relations between the parties of SPPhS effective, and this will solve all issues on SP between a PhS, an employer and the executive authority in the optimal way. Settlement of conflicts between a PhS, an employer and the executive authority requires assigning of an authorized person who is responsible for social issues (APSI) at the labour collective of pharmaceutical institutions of all types of ownership and determining qualification requirements for the staffing.

4. The studies conducted have shown that there is a need to reconsider the current LP system in the pharmaceutical industry of Ukraine and to reform the LP system for pharmaceutical wholesale and retail branch.

REFERENCES

1. Конституція України // Офіційний вісник України. – 2010. – №72/І. – 2598 с.
2. Толочко В.М., Зарічкова М.В. // Укр. вісник психоневрол. – 2013. – Т. 21, вип. 2 (75). – С. 136-137.

3. Толочко В.М., Зарічкова М.В. // Вісник фармації. – 2012. – №2. – С. 37-39.
4. Толочко В.М., Зарічкова М.В. Інформ. лист. – Х.: Вид-во НФаУ, 2013. – 3 с.
5. Bazeley P. // Res. in the schools. – 2006. – Vol. 13, №1. – P. 64-74. [Електронний ресурс]. – Режим доступу до журн.: http://www.msersa.org/Rits_131/Bazeley_131.pdf.
6. Chiang H., Goes P., Stohr E. // ACM Transaction on management information systems. – 2012. – Vol. 3, №3. – P. 12-25. [Електронний ресурс]. – Режим доступу до журн.: http://www.informationintelligence.org/Articles/Business_Intelligence_and_Analytics_Education_ACM_Oct_2012.pdf.
7. Driscoll L., Appiah-Yeboah A., Salib P., Rupert D. // Ecol. and Environmental Anthropol. – 2007. – Vol. 3, №1. – P. 19-28. [Електронний ресурс]. – Режим доступу до журн.: <http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1012&context=icwdmee>
8. European social Policy Forum. Brussels, 24-26 June. 1998. Summary report / Ed. by M.Carley. – №29, 28, 88.
9. Imai K., King G., Lau O. // J. of computational and graphical statistics. – 2008. – Vol. 17, №4. – P. 1-22. – DOI:10.1198/106186008X384898. – [Електронний ресурс]. – Режим доступу до журн.: <http://gking.harvard.edu/files/z.pdf>.
10. Tolochko V., Zarichkova M., Medvedyeva Y., Tolochko K. // Intern. J. of Pharmac. Sci. Review and Res. – 2013. – Vol. 18, Issue 1, January – February. [Електронний ресурс]. – Режим доступу: <http://www.globalresearchonline.net/pharmajournal/vol18iss1.aspx>.
11. Wickham H. // J. of statistical software. – 2011. – Vol. 40, №1. – P. 1-29. [Електронний ресурс]. – Режим доступу до журн.: <http://www.jstatsoft.org/v40/i01/paper>.

АНАЛІЗ СТАВЛЕННЯ СПЕЦІАЛІСТІВ ФАРМАЦІЇ ДО ІСНУЮЧОЇ СИСТЕМИ СОЦІАЛЬНОГО ЗАХИСТУ ТА НАПРЯМКИ ЇЇ РЕФОРМУВАННЯ

М.В.Зарічкова

Ключові слова: соціальний захист спеціалістів фармації; соціальні послуги; фармацевтична галузь; уповноважена особа з соціальних питань; охорона праці; комп'ютерна програма ADPQ_(SPPhS)

Проаналізований соціальний статус спеціалістів фармації (СФ) оптово-роздрібної ланки фармацевтичної галузі України та їх ставлення до існуючої системи соціального захисту (СЗ). Досліджені можливості СФ щодо отримання соціальної допомоги за місцем роботи, участі у соціальних програмах та пропозицій до реформування існуючої системи СЗ. Встановлено необхідність підвищення ролі громадських та самоврядних організацій в системі соціального захисту спеціалістів фармації (СЗСФ), що дозволить покращити відносини між учасниками СЗСФ та дієво впливати на вирішення конфліктних ситуацій між СФ та роботодавцем. Для цього запропоновано введення в трудовий колектив фармацевтичних закладів усіх форм власності уповноваженої особи з соціальних питань. Уперше розроблена комп'ютерна програма ADPQ_(SPPhS) (Automatic data processing questionnaires (attitude to an existing social protection for pharmacy specialists (SPPhS)) для обробки результатів дослідження СЗСФ оптово-роздрібної ланки фармацевтичної галузі України.

АНАЛИЗ ОТНОШЕНИЯ СПЕЦИАЛИСТОВ ФАРМАЦИИ К СУЩЕСТВУЮЩЕЙ СИСТЕМЕ СОЦИАЛЬНОЙ ЗАЩИТЫ И ПУТИ ЕЕ РЕФОРМИРОВАНИЯ

М.В.Заричкова

Ключевые слова: социальная защита специалистов фармации; социальные услуги; фармацевтическая отрасль; уполномоченное лицо по социальным вопросам; охрана труда; компьютерная программа ADPQ_(SPPhS)

Проанализирован социальный статус специалистов фармации (СФ) оптово-розничного звена фармацевтической отрасли Украины и их отношение к существующей системе социальной защиты (СЗ). Исследованы возможности СФ относительно получения социальной помощи по месту работы, участия в социальных программах и предложений к реформированию существующей системы СЗ. Установлена необходимость повышения роли общественных и организаций самоуправления в системе социальной защиты специалистов фармации (СЗСФ), что позволит улучшить отношения между участниками СЗСФ и действительно влиять на решение конфликтных ситуаций между СФ и работодателем. Для этого предложено введение в трудовой коллектив фармацевтических учреждений всех форм собственности уполномоченного лица по социальным вопросам. Впервые разработана компьютерная программа ADPQ_(SPPhS) (Automatic data processing questionnaires (attitude to an existing social protection for pharmacy specialists (SPPhS)) для обработки результатов исследования СЗСФ оптово-розничного звена фармацевтической отрасли Украины.

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

UDC 615.015.32

THE STUDY OF EXTEMPORANEOUS COMPOUNDING OF HOMEOPATHIC MEDICINAL PRODUCTS

V.M.Tolochko, D.V.Vakulenko, I.V.Shyshkina

National University of Pharmacy

Key words: extemporaneous compounding; homeopathic medicinal products; allopathic medicinal products; pharmaceutical formulations

The studies have shown that the manufactured homeopathic medicinal products (MP) existing at the current domestic pharmaceutical market are not appropriate for the individual approach of homeopathic treatment, so the need in their extemporaneous compounding is pressing. One of the ways to solve this problem is expansion of the chain of specialized homeopathic pharmacies and departments on the basis of the existing pharmacies of the general profile. To implement this method, the specificity of the production function of pharmacies (departments), which is based on extemporaneous compounding of homeopathic MP, has been investigated. It has been found that the proportion of extemporaneous compounding of homeopathic MP by means of intrapharmacy products among the total number of extemporaneous compounding of homeopathic MP in specialized pharmacies constitutes 89.79%, and in pharmacies of the general profile (homeopathic department) it is 14.04%. It is important to mention that the proportion of funds for implementation of homeopathic MP in the total cost of medicines sold by pharmacies according to prescriptions of extemporaneous compounding is higher than 50%. It is based on preparation and sale of solid, liquid and soft medicinal dosage forms of 77.16%, 14.37% and 8.74%, respectively; and it proves the expedience of creating such specialized pharmacies (departments).

The priority for use of homeopathic treatment is an individual approach to each patient, and it is possible by disposal of homeopathic medicinal products (MP) from pharmacies under prescriptions of homeopaths. The conclusions of the study of availability of homeopathic MP at the domestic pharmaceutical market were given in our previous studies. They indicate that offers of foreign manufacturers are prevailing. However, they do not satisfy completely the need in the individual approach to treatment [6, 11]. When choosing homeopathy as a method of treatment, extemporaneous compounding of homeopathic MP is important and necessary. These medicines are of high quality and available at their price [4, 9]. Therefore, within the current economic climate in the country, it is important to increase extemporaneous compounding of homeopathic MP under the terms of both specialized homeopathic pharmacies and departments on the basis of the existing pharmacies of the general profile, which have material support and are eligible to produce products according to the current license provisions. The practicability of homeopathic MP compounding within the pharmacy conditions has been also confirmed by the results of opinion surveys conducted by us among the population of some regions of Ukraine [6, 11].

The aim of our work was to determine the production process output and its characteristics. This is possible due to the study of extemporaneous compounding of homeopathic MP.

Materials and Methods

Personal conclusions of the study of specialized homeopathic and allopathic pharmacies producing extemporaneous homeopathic MP in Odessa and Poltava re-

gions were used in the research. More than 650 stopwatch readings were conducted; more than 800 prescription formulations for the period of 2013-2014 were studied. 71.0% of them were formulations for homeopathic MP.

In our study the following research methods were used: historical, comparison, logical, analytical analysis, statistical, interviews, questionnaires, direct supervision, stopwatch study, work time study. The results were processed by computer programmes and presented graphically. Regulatory documents of the Ministry of Public Health of Ukraine concerning the production regulation and drug quality control in pharmacies were used in the studies [1, 2, 3, 4, 5, 7, 8, 10].

Results and Discussion

Analysis of extemporaneous compounding of homeopathic MP has shown that it is divided into repetitive and individual formulations. Therefore, much attention is paid to production of the intrapharmacy products for repeated formulations in some pharmacies under research. This step makes it possible to reduce time for dispensing a drug by prescription and to reduce the cost of MP per each formulation. However, the possibilities for production of intrapharmacy products in specialized homeopathic pharmacies and pharmacies of the general profile, which prepare homeopathic MP prescribed by homeopaths, are not the same. Therefore, there is a need for the constant study of formulations to determine the repeated ones and for further recommendations to use them in preparing intrapharmacy products. It has been found that this work is organized better in specialized homeopathic pharmacies.

Table 1

The structure of extemporaneous compounding of homeopathic MP

The sale of homeopathic MP of extemporaneous compounding	Specialized homeopathic pharmacies		Pharmacies of the general profile with the homeopathic department	
	Annual average number of prescriptions			
	Absolute index	%	Absolute index	%
Intrapharmacy products according to the repeated formulations	14352	89.79	1176	14.04
MP produced according to individual prescriptions	1632	10.21	7200	85.96
Total:	15984	100.0	8376	100.0

As shown in Table 1, the proportion of extemporaneous compounding of homeopathic MP due to intrapharmacy products among the total number of extemporaneous prescriptions for homeopathic MP in specialized pharmacies constitutes 89.79%, and in pharmacies of the general profile (homeopathic department) it is only 14.04%.

The abovementioned facts indicate that more attention should be paid to development of specialized homeopathic pharmacies while expanding the pharmacy chain to provide patients with homeopathic MP prepared extemporaneously. It is clear that this approach requires considerable time and money, so development of homeopathic departments in pharmacies of the general profile is also promising. To confirm this information we studied additionally some aspects of extemporaneous prescriptions of homeopathic departments. For this purpose, the analysis of extemporaneous prescriptions received by the homeopathic department of the pharmacy of the general profile within a month was conducted. It has been found that these prescriptions for homeopathic MP are received regularly, their daily number ranges from 1 to 55. Thus, the homeopathic department has a daily load of extemporaneous compounding of homeopathic MP (Fig. 1).

It is important to mention that under these conditions the proportion of funds for dispensing homeopathic MP in the total cost of medicines sold by a pharma-

cy by prescriptions of extemporaneous compounding is higher than 50% and it ranges from 29.0% to 85.0% within a month (Fig. 2). In other words, there is an economic efficiency for specialized homeopathic pharmacies (departments) when introducing extemporaneous compounding of homeopathic MP.

It is known that each medicinal dosage form of extemporaneous compounding has its own production technology; and it is also important for extemporaneous homeopathic MP [4]. Extemporaneous compounding of homeopathic MP is presented by solid, liquid and soft medicinal dosage forms. Therefore, the next step of our study was the comparison of the ratio of different medicinal dosage forms in specialized homeopathic pharmacies and departments in pharmacies of the general profile (Tab. 2).

Table 2 shows that the ratio of different medicinal dosage forms of homeopathic MP in specialized pharmacies and pharmacies of the general profile (homeopathic department) is almost the same. It has been determined that solid medicinal dosage forms prevail – 72.37% and 81.96%, respectively, on average – 74.16%. Rubbing (powders, trituration), granules (globs, diabetes pills) are among them. Globes are the most common.

As for the ratio of liquid medicinal dosage forms in extemporaneous compounding of homeopathic MP, they are 17.54% and 11.19%, respectively, on average – 14.37%. These medicinal dosage forms include formu-

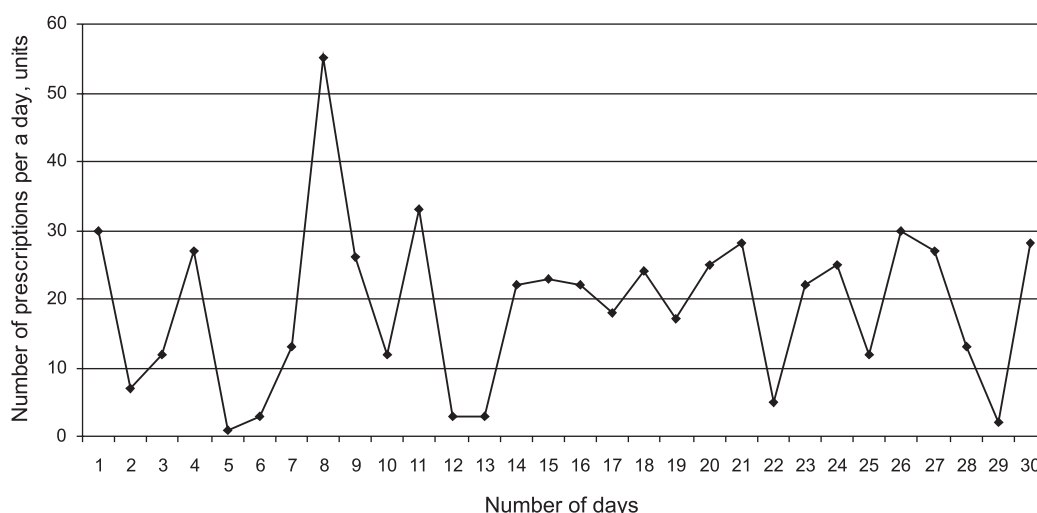


Fig. 1. The average number of prescriptions for extemporaneous compounding of homeopathic MP within a month in the homeopathic department of a pharmacy of the general profile.

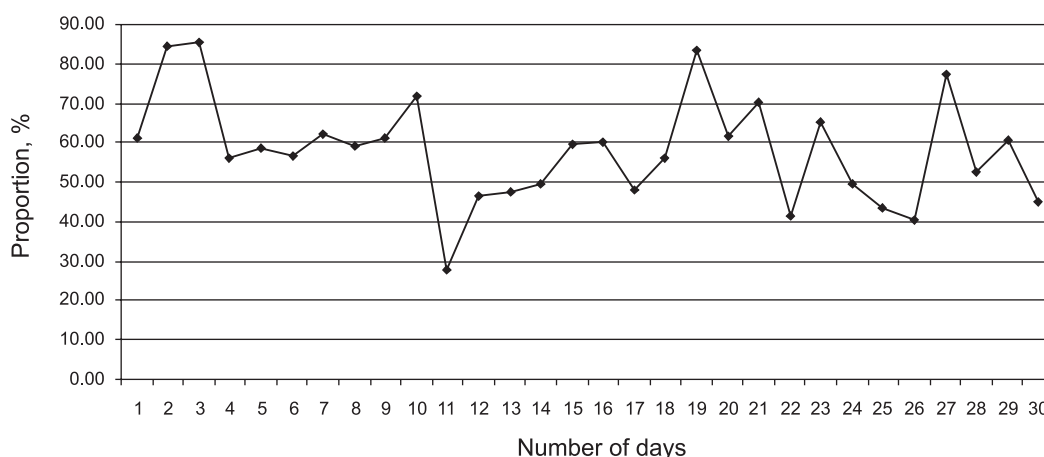


Fig. 2. Proportion of the cost of homeopathic MP of extemporaneous compounding in the total cost of extemporaneous MP sold by a pharmacy of the general profile.

Table 2

The ratio of medicinal dosage forms in extemporaneous compounding of homeopathic MP (%)

Medicinal dosage forms	Specialized homeopathic pharmacy	Pharmacy of the general profile with the homeopathic department	Average
Solid	72.37	81.96	77.16
Liquid	17.54	11.19	14.37
Soft	10.09	6.85	8.47
Total:	100.0	100.0	100.0

lations for internal (mixture, drops) and external (alcohols, oils, liniments) use.

Soft medicinal forms constitute 10.09% and 6.85% in total, on average – 8.47%. It has been determined that ointments (balms, suspension ointments, emulsion creams, combined ointments) and suppositories are among them.

It has been also found that by the complexity (the number of components), extemporaneous compounding of homeopathic MP consists of one-component or three-component formulations in 80% of cases. This is due to the fact that one-component medicines (monodrugs) are received by prescriptions of homeopaths in different dilutions (potencies), they create favourable conditions for individual approach in homeopathy.

CONCLUSIONS

1. Extemporaneous compounding of homeopathic MP in the pharmacy conditions is pressing at the current domestic pharmaceutical market. It requires the fur-

ther development by expanding the chain of specialized homeopathic pharmacies and homeopathic departments on the basis of the existing pharmacies of the general profile.

2. The study of extemporaneous compounding of homeopathic MP conducted has allowed to determine its characteristics under the conditions of specialized homeopathic pharmacies (departments). It is associated with the ratio between intrapharmacy products and individual prescriptions, their daily number, the availability of solid, liquid and soft medicinal dosage forms, domination of one-component formulations over three-component ones.

3. The economic efficiency of homeopathic MP of extemporaneous compounding at the pharmaceutical market both for patients (availability) and pharmacies has been proven; it has been confirmed by the proportion of their costs in the total cost of extemporaneous MP of the general profile and it is up to 85.0%.

REFERENCES

1. Грошовий Т.А., Марценюк В.П., Кучеренко Л.І. та ін. Математичне планування експерименту при проведенні наукових досліджень у фармації: Підруч. – Тернопіль: ТДМУ, 2008. – 368 с.
2. Закон України «Про лікарські засоби». [Електрон. ресурс]. – Режим доступу: <http://zakon4.rada.gov.ua/laws/show/про%20лікарські%20засоби>.
3. Наказ «Про затвердження Інструкції про порядок контролю якості лікарських засобів під час оптової та роздрібної торгівлі». [Електрон. ресурс]. – Режим доступу: <http://zakon3.rada.gov.ua/laws/show/z0107-02>.
4. Основы гомеопатической фармации: Учеб. для фармац. вузов / Под ред. А.И.Тихонова. – Х.: НФАУ; Золотые страницы, 2002. – 576 с.

5. Пархоменко В.М. Методи вибіркового обстеження: Навч. посіб. – К.: 2001. – 148 с.
6. Толочко В.М., Вакуленко Д.В. // Фармац. журн. – 2014. – №2. – С. 13-19.
7. Code of Federal Regulations Parts 210 and 211: Current good manufacturing practice in manufacturing, processing, packing or holding of drugs; general and current good manufacturing practice for finished pharmaceuticals. Rockville, MD, US Food and Drug Administration, 2006. [Електрон. ресурс]. – Режим доступу: <http://www.fda.gov/downloads/Drugs/.../Guidances/ucm070342.pdf>
8. Hahnemann Laboratories, Inc. [Електрон. ресурс]. – Режим доступу: <http://www.hahnemannlabs.com/preparation.html>
9. <http://www.who.int/medicines/areas/traditional/Homeopathy.pdf>
10. Kayne S.B. Homeopathic Pharmacy: Theory and Practice. – Ilsevier Churchill Livingstone, 2006. – P. 386.
11. Safety issues in the preparation of homeopathic medicines. [Електрон. ресурс]. – Режим доступу: <http://www.who.int/medicines/areas/traditional/Homeopathy.pdf>
12. Tolochko V.M., Vakulenko D.V. // Матер. за IX Междунар. научна практ. конф. «Научный потенциал на света – 2013». Т. 15 «Лекарство». София: «БелГРАД – БГ» ООД, 2013. – С. 12-13.

ВИВЧЕННЯ ЕКСТЕМПОРАЛЬНОЇ РЕЦЕПТУРИ ГОМЕОПАТИЧНИХ ЛІКАРСЬКИХ ЗАСОБІВ

В.М.Толочко, Д.В.Вакуленко, І.В.Шишкіна

Ключові слова: екстемпоральна рецептура; гомеопатичні лікарські засоби; алопатичні лікарські засоби; лікарські форми

Визначено, що присутні на сучасному вітчизняному фармацевтичному ринку гомеопатичні ЛЗ промислового виробництва повністю не задовольняють індивідуальний підхід гомеопатичного методу лікування, тому потреба в збільшенні їх екстемпорального виробництва є актуальною. Одним із напрямків вирішення цієї проблеми є розширення мережі спеціалізованих гомеопатичних аптек та відділів на базі діючих аптек загального профілю. Для реалізації такого напрямку досліджена специфіка виробничої функції аптек (відділів), яка базується на екстемпоральній рецептурі гомеопатичних ЛЗ. З'ясовано, що питома вага екстемпоральної рецептури гомеопатичних ЛЗ за рахунок внутрішньоаптечних заготовок серед загальної кількості екстемпоральних рецептів на гомеопатичні ЛЗ у спеціалізованих аптеках сягає 89,79%, а в аптеках загального профілю (гомеопатичний відділ) складає 14,04%. Важливо зауважити, що питома вага коштів за реалізацію гомеопатичних ЛЗ в загальній вартості відпущених з аптеки ЛЗ за рецептами екстемпорального виготовлення складає понад 50% і базується на виготовленні й відпуску твердих, рідких і м'яких лікарських форм, відповідно, 77,16%, 14,37% і 8,74%, що обґрунтовує доцільність створення таких спеціалізованих аптек (відділів).

ИЗУЧЕНИЕ ЭКСТЕМПОРАЛЬНОЙ РЕЦЕПТУРЫ ГОМЕОПАТИЧЕСКИХ ЛЕКАРСТВЕННЫХ СРЕДСТВ

В.М.Толочко, Д.В.Вакуленко, И.В.Шишкіна

Ключевые слова: экстемпоральная рецептура; гомеопатические лекарственные средства; аллопатические лекарственные средства; лекарственные формы

Исследования показали, что присутствующие на современном отечественном фармацевтическом рынке гомеопатические ЛС промышленного производства в достаточной мере не удовлетворяют индивидуальный подход гомеопатического метода лечения, поэтому потребность в увеличении их экстемпорального производства является актуальной. Одним из направлений решения этой потребности является расширение сети специализированных гомеопатических аптек и отделов на базе действующих аптек общего профиля. Для реализации такого направления исследована специфика производственной функции аптек (отделов), которая базируется на экстемпоральной рецептуре гомеопатических ЛС. Выяснено, что удельный вес экстемпоральной рецептуры гомеопатических ЛС за счет внутриаптечных заготовок среди общего количества экстемпоральных рецептов на гомеопатические ЛС в специализированных аптеках достигает 89,79%, а в аптеках общего профиля (гомеопатический отдел) составляет 14,04%. Важно обратить внимание на то, что удельный вес средств от реализации гомеопатических ЛС в общей стоимости отпущенных из аптеки ЛС по рецептам экстемпорального изготовления составляет более 50% и базируется на изготовлении и отпуске твердых, жидких и мягких лекарственных форм, соответственно, 77,16%, 14,37% и 8,74%, что обосновывает целесообразность создания таких специализированных аптек (отделов).

ЕКСПЕРИМЕНТАЛЬНА ТА КЛІНІЧНА ФАРМАКОЛОГІЯ

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

UDC 615.322:615.451.16:615.213

ANTIEPILEPTIC POTENTIAL OF *FUMARIA SCHLEICHERI* AND *OCIMUM BASILICUM* DRY EXTRACTS

V.V.Tsyvunin, S.Yu.Shtrygol'

National University of Pharmacy

Key words: medicinal plants; extracts; antiepileptic drugs

*The results of investigation of the antiepileptic activity of promising herbal anticonvulsants – *Fumaria schleicheri* (FSDE) and *Ocimum basilicum* (OBDE) dry extracts – are presented. The herbal remedies were administered intragastrically in the conditionally effective dose of 100 mg/kg in the therapeutic and preventive mode during 3 days with the last time of 30 minutes before the experiments. As reference drugs sodium valproate and carbamazepine were chosen. Under the conditions of maximal electroshock (MES) test the ability of the chosen dry extracts to prevent the development of primarily generalized convulsions was studied. For the in-depth study of antiepileptic properties of FSDE and OBDE the model of pentylenetetrazole (corasole)-induced kindling has been chosen. In general the researched dry extracts in the condition of the MES test have shown the anticonvulsant effect on the level of sodium valproate, but they were slightly inferior in efficacy compared with carbamazepine. On the model of pentylenetetrazole-induced kindling it has been shown that FSDE unlike OBDE and sodium valproate has the ability to prevent convulsions under the conditions of experimental chronic epileptogenesis. Thus, it has been found that OBDE is able to prevent the development of primary generalized seizures, while FSDE prevents acute paroxysms stimulated by electrical impulse and chronic epileptogenesis.*

Under conditions of progressing development and spreading of nervous and psychic diseases the problem of improving the quality of treating epilepsy is topical. According to the data of the WHO almost 0.68 per cent of the world population suffers from this disease and this figure has been increasing steadily [11]. It is well known that treating chronic diseases, including epilepsy, is a rather long-term if not lifelong process [3]. That is why in this case the use of herbal medicines is relevant as they are highly safe even in the situation of a long-term application [12].

In the previous studies on different models of chemo-induced convulsions, including those caused by pentylenetetrazole (the main screening model [1]), picrotoxin, thiosemicarbazide, strychnine and camphor, the high level of anticonvulsant properties was shown by the dry extract of fumitory (*Fumaria schleicheri* Soy.-Willem., *Fumariaceae*) and the dry extract of basil (*Ocimum basilicum* L., *Lamiaceae*) [7, 8]. Taking into account the absence of herbal medicines with the proven anticonvulsant activity at the domestic and international pharmaceutical markets the profound study of the antiepileptic properties of these dry extracts is reasonable.

The aim of this work is to study the antiepileptic potential of the promising herbal anticonvulsant drugs – dry extracts of *Fumaria schleicheri* (FSDE) and *Ocimum basilicum* (OBDE) obtained from the aerial part

of the plants according to the requirements of the State Pharmacopoeia of Ukraine – taking into consideration their ability to prevent primarily generalized convulsions on the model of seizures induced by the maximal electroshock and the ability to inhibit epileptogenesis under conditions of pentylenetetrazole-induced kindling.

Materials and Methods

The experimental part was performed on 86 white random-bred mice. The animals were kept in the standard conditions of the vivarium of the Central Research Laboratory at the National University of Pharmacy according to the hygiene norms and principles of the European Convention on laboratory animals protection (Strasbourg, 1986). In the research period the animals were kept in the vivarium at the temperature of 19-24°C, with humidity of not more than 50%, in the “day-night” natural light mode in plastic cages with the standard nutrition and free access to water and food. All research processes were performed according to the “General ethical principles of the experiments with animals” [6] and according to the methodological recommendations for pre-clinical study of specific activity of potential anticonvulsant medicines [1].

In the conditions of maximal electroshock (MES) test the ability of the chosen dry extracts to prevent the development of primarily generalized convulsions was studied [1]. The experiments were conducted on 55 random-

bred male albino mice with the body weight of 21-27 g that were randomly divided into 5 groups: 1 – control; 2-3 – the groups where animals received aqueous solutions of the given dry extracts; 4-5 – comparison groups where animals received reference drugs such as sodium valproate and carbamazepine. The reference drugs were chosen taking into account the recommendations [1, 4].

The dry extracts studied were obtained by Yu.S. Prokopenko, the assistant of the Department of Quality, Standardization and Certification of Medicines, the Institute of Pharmacy Professionals Qualification Improvement at the National University of Pharmacy. The aerial parts of *Fumaria schleicheri* Soy.-Willem. and *Ocimum basilicum* L. were collected in the phase of blooming in different regions of Ukraine (Kharkiv, Luhansk, Donetsk, Ternopil, Rivne, Zhytomyr, Kyiv regions and the Republic of Crimea). The raw material was washed with water and air-dried. The dried material was kept at the room temperature. A relatively dry herbal material was powdered and extracted with distilled water (1:10) for 2 hours at the temperature of 80°C. The process was repeated 3-4 times up to the full extraction of the biologically active substances from the raw material. The received extracts were mixed, filtered and concentrated in vacuum device at the temperature of 50-60 °C and the pressure of 80-87 kPa up to thick consistency. The received semi-product was dried in vacuum-drying device up to the remaining humidity of 5% [5].

FSDE was standardized according to the content of alkaloids (the noscapine group) and flavonoids (flavonoles and flavons) using the method of absorption UV-spectrophotometry [5], and OBDE – according to the content of flavonoids.

The animals of experimental group received intragastrically the aqueous solutions of the given dry extracts in the conditionally effective dose 100 mg/kg [7] in the treating and preventive mode during 3 days with the last time 30 minutes before conducting the experiment. The comparison groups received intragastrically classic antiepileptic medicines – sodium valproate (the syrup “Depakine”, Sanofi-Aventis, France) in the dose of 300 mg/kg and carbamazepine (“Finlepsin”, TEVA, Israel/Poland) in the dose of 40 mg/kg in the same mode. The second reference drug was injected in the form of a thin aqueous suspension solubilized by Tween-80. The mice in the control group received intragastrically distilled water (0.1 ml per 10 g of the body weight).

Then the animals through the corneal electrodes were affected by electric stimuli with duration of 0.2 sec, frequency of 50 Hz and current of 50 mA. Under these conditions 100% of animals in the control group had maximum tonic extension of hind limbs. The anticonvulsant action was estimated according to the following indicators: duration of convulsions, the time of the survived mice recovery, the time of the animals' death and the percentage of lethality in the experimental groups [4].

For the profound study of antiepileptic properties of the extracts the model of pentylene-tetrazole (corasole)-induced kindling was chosen [1, 4].

Kindling is a phenomenon based on the occurrence of focal convulsion afterdischarge, behavioral automatisms and generalized convulsion attacks in response to multiple epileptic stimulations of subthreshold intensity that at first do not cause convulsions. The kindling model is characterized with the unique methodological advantages for experimental determination of the effect of potential anticonvulsant drugs on pathophysiology of seizures [1, 4]. Under conditions of the kindling model one can clearly check the start of convulsions, the stages of their development and preserving. Behavioral patterns in this model are characterized by high repeatability, easy visual control of their intensity, severity and duration. The kindling model reflects pathophysiological and clinical specificities of epilepsy in the most adequate way and the phenomenon of “swaying” is viewed as an universal mechanism, which takes part in epilepsy not only experimentally, but it is also practically characteristic for the human brain [9].

For the experiment 31 random-bred albino male mice with the body weight of 22-29 g were selected. The animals were divided into 4 groups (n=7-9): 1– control; 2-3 – groups of animals that received aqueous solutions of the given dry extracts; 4 – group of comparison that received sodium valproate as a reference drug.

The animals of experimental groups received intragastrically the aqueous solutions of appropriate dry extracts in the conditionally therapeutic dose of 100 mg/kg [7] in the therapeutic and preventive mode during 27 days once a day 30 minutes before injecting the convulsant. The group of comparison received intragastrically a classic anticonvulsant drug sodium valproate (the syrup “Depakine”, Sanofi-Aventis, France) in the dose of 300 mg/kg in the same mode. The mice from the control group received intragastrically distilled water (0.1 ml per 10 g of the body weight). The aqueous solution of pentylene-tetrazole (corasole) was injected intraperitoneally in the subthreshold dose of 30 mg/kg.

After injecting of the convulsant every mouse was placed into a separate plastic cylinder box with the diameter of 20 cm and the height of 35 cm. The state of every mouse was being observed for 30 min. The anticonvulsant activity was estimated daily according to the following indicators: the percentage of mice with convulsions in each group, the number of days with paroxysms in the group, as well as according to the most informative indicator – the day of occurrence of the first convulsions in the group [4].

The results was expressed as mean \pm standard error of mean (SEM). Statistical differences between groups were analyzed using Student's t-test (in case of normal distribution), Mann-Whitney U test and Fisher angular transformation (for the alternative form of analysis). The level of statistical significance was considered as $p < 0.05$.

Results and Discussion

The maximal electroshock in 100% of animals from the control group was followed by immediate development of several convulsions in the form of clonic-tonic paroxysms with prevailing of the tonic component. The

Table

The effect of *Fumaria schleicheri* and *Ocimum basilicum* dry extracts, sodium valproate and carbamazepine on the seizures induced by the maximal electroshock in mice ($M \pm m$)

Animal Group	Dose, mg/kg	n	Duration of convulsions, sec	Time of recovery, sec	Time of death, sec	Lethality, %
Control	–	15	19.40±3.66	18.71±7.89	20.00±1.85	53.3
FSDE	100	10	9.20±2.28*/**	6.14±1.68**	16.33±4.70	30*****
OBDE	100	10	9.88±3.47**	1.83±0.98*#	21.75±2.93	40*****
Sodium valproate	300	10	8.60±2.37*/**	4.29±0.47**	18.67±3.18	30*****
Carbamazepine	40	10	1.70±1.07****#	1.70±1.07*	–	0***

Note: 1. Statistically significant differences: * – compared to control ($p < 0.05$); ** – compared to the group receiving carbamazepine ($p < 0.05$); *** – compared to control ($p < 0.01$); **** – compared to control ($p < 0.001$); ***** – compared to the group receiving carbamazepine ($p < 0.01$); # – compared to the group receiving sodium valproate ($p < 0.05$).

2. Abbreviations: FSDE – *Fumaria Schleicheri* dry extract; OBDE – *Ocimum Basilicum* dry extract.

death of 53.3% of mice was caused by the tonic extension of hind limbs (Table). It corresponds to the existing data, which show the variation of the lethality level in the wide range from 0 to 100% [10, 13] depending on many factors.

In the MES test the herbal drugs studied showed marked anticonvulsant properties. FSDE decreased duration of convulsions by 2.1 times compared to control ($p < 0.05$), and also decreased the time of recovery of the survived animals by 3 times ($p > 0.05$). Such indicators as the time of death and the lethality level in the group were not seriously influenced by FSDE.

OBDE decreased duration of the tonic extension of hind limbs almost by 2 times compared to the control group, but because of the high dispersion of data this difference did not reach the level of statistical significance. But OBDE significantly decreased the time of the mice recovery by more than 10 times compared to the indicator in the control group. The positive effect of OBDE on the time of death and lethality in the experimental group was not recorded.

Sodium valproate showed the effect at the level of the studied dry extracts – it statistically significantly de-

creased the duration of convulsions compared with the control group by 2.3 times and showed the tendency to decrease the recovery time by 4.3 times without the influence on the death time and lethality level in the group.

Carbamazepine showed the most expressed anticonvulsant activity, which significantly exceeded the similar effect of the studied herbal drugs and sodium valproate. Under its action not only duration of convulsions, but also the recovery time decreased by 11.4 and 11.0 times, respectively. Besides, carbamazepine completely prevented the death of animals ($p < 0.01$).

On the model of pentylenetetrazole-induced kindling according to the most informative indicator – the day of the first convulsions occurrence – FSDE continued the latent period of the first convulsions occurrence with statistical significance (Fig. 1): in the control group spontaneous seizures were observed on the 23rd day of the study, while FSDE continued the latent period of attack occurrence up to the 27th day ($p < 0.01$).

According to the data of Fig. 1 it can also be observed that FSDE decreased the percentage of mice with convulsions in the group from the 23rd to the 27th day,

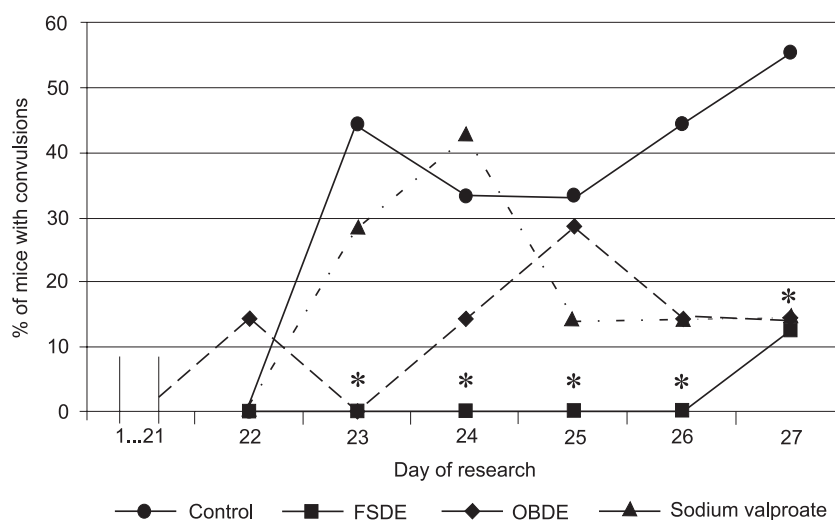


Fig. 1. Dynamics of the anticonvulsant activity of *Fumaria schleicheri* and *Ocimum basilicum* dry extracts and sodium valproate under conditions of pentylenetetrazole-induced kindling.

Note: * – statistically significant difference compared to control ($p < 0.05$).

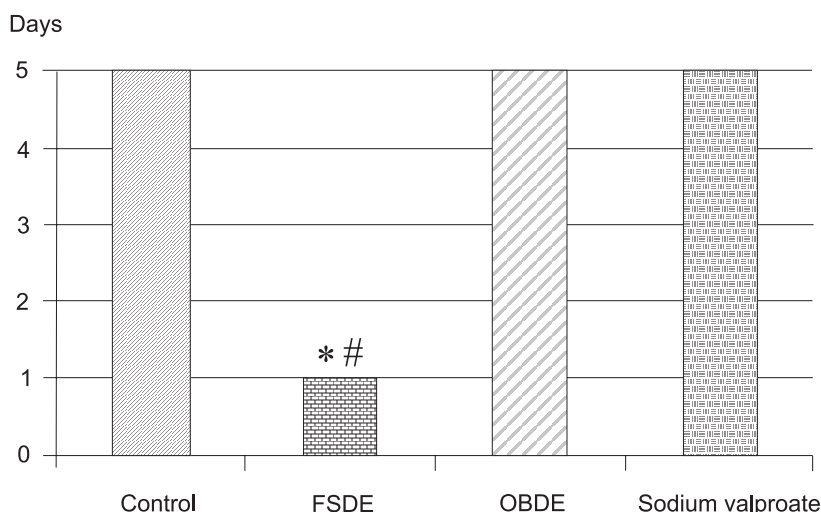


Fig. 2. The number of days with convulsions in the experimental groups on the model of pentylenetetrazole-induced kindling.
Note. Statistically significant difference: * – compared to control ($p < 0.01$); # – compared to sodium valproate ($p < 0.01$).

including the last one ($p < 0.05$), while OBDE at the same dose decreased the corresponding indicator only on the 23rd and the 27th days. The reference drug sodium valproate on this model showed relatively weak antiepileptic properties: significant decrease of the percentage of mice with convulsions was observed only on the 27th day of the study. Besides, in the groups where animals received OBDE and sodium valproate no increase of the latent period of spontaneous seizures occurrence was observed.

As can be seen from the data of Fig. 2, only FSDE significantly decreased the total number of days with convulsions compared to control ($p < 0.01$). Neither OBDE, nor sodium valproate affected this indicator under the same conditions.

As it is known, the anticonvulsant properties are characteristic to combinations of different chemical compounds [2, 12]. However, most representatives with the above-mentioned pharmacological activity belong to such classes of biologically active substances as flavonoids, alkaloids, as well as terpenes and volatile components of essential oils [2]. The role of individual components

and fractions of FSDE and OBDE in providing the anticonvulsive effect requires further verification and is a subject of the next research stage.

CONCLUSIONS

1. The antiepileptic properties of *Fumaria schlei-cheri* dry extract (FSDE) and *Ocimum basilicum* dry extract (OBDE) have been studied.

2. Under conditions of the maximal electroshock test it has been found that FSDE and OBDE in the dose of 100 mg/kg showed considerable anticonvulsant properties that were not inferior to the effect of sodium valproate in the dose of 300 mg/kg, but did not reach the level of carbamazepine in the dose of 40 mg/kg.

3. On the model of pentylenetetrazole-induced kindling it has been shown that FSDE unlike OBDE and sodium valproate has the ability to prevent convulsions under conditions of the experimental chronic epileptogenesis.

4. The obtained data allow recommending FSDE for further research with the aim of developing the first Ukrainian herbal antiepileptic drug.

REFERENCES

1. Головенко М.Я., Громов Л.О. Доклінічне вивчення специфічної активності потенційних протисудомних препаратів: Метод. рекоменд. – К.: Авіценна, 2003. – 26 с.
2. Данилов С.А., Товчига О.В., Штриголь С.Ю., Степанова С.І. // Фармаком. – 2011. – №4. – С. 68-87.
3. Марценковский И.А. // Здоров'я України. – 2006. – №23. – С. 43-45.
4. Миронов А.Н. Руководство по проведению доклинических исследований лекарственных средств. Ч. 1. – М.: Гриф и К, 2012. – 944 с.
5. Пат. UA 89372 на кор. модель, МПК А 61 Р 25/22 (2006.01), А 61 К 35/50 (2006.01), А 61 К 135/00 (2006.01) / Лікувально-профілактичний засіб з анксиолітичною дією на основі рослинної сировини / В.В.Цивунін, Ю.С.Прокопенко, С.Ю.Штриголь, В.А.Георгіяну. – № и 2013 04354. – Заявл.: 08.04.2013. Опубл.: 25.04.2014. – 2014. – Бюл. №8. – 6 с.
6. Стефанов О.В. Доклінічні дослідження лікарських засобів: Метод. рекоменд. – К., 2001. – 527 с.
7. Цивунін В.В., Штриголь С.Ю., Прокопенко Ю.С., Георгіяну В.А. // Клінічна фармація. – 2012. – Т. 16, №4. – С. 47-50.
8. Цивунін В.В., Штриголь С.Ю., Прокопенко Ю.С., Торянник Е.Л. // УБФЖ. – 2014. – №3 (32). – С. 45-49.
9. Шандра А.А., Коп'єва Н.В. // Вісник психіатрії та психофармакотерапії. – 2008. – №2 (14). – С. 7-17.

10. Штрыголь С.Ю. Модуляция фармакологических эффектов при различных солевых режимах: Монография. – Х.: Ависта-ВЛТ, 2007. – 360 с.
11. Эпилепсия. Информац. бюл. №999 [Электронный ресурс] / Всемирная организация здравоохранения [сайт]. – Режим доступа: <http://www.who.int/mediacentre/factsheets/fs999/ru/> (16.05.2014). – Загл. с экрана.
12. Ngo Bum E. // *Clinical and Genetic Aspects of Epilepsy*. – 2011. – P. 175-192.
13. Silambujanaki P., Chitra V., Suman K. et al. // *Res. J. of Pharmac., Biol. and Chem. Sci.* – 2010. – Vol. 1, №2. – P. 431-435.

ПРОТИЕПІЛЕПТИЧНИЙ ПОТЕНЦІАЛ СУХИХ ЕКСТРАКТІВ РУТКИ ШЛЕЙХЕРА ТА БАЗИЛІКУ КАМФОРНОГО

В.В.Цивунін, С.Ю.Штрыголь

Ключові слова: лікарські рослини; екстракти; протиепілептичні засоби

В роботі представлені результати дослідження протиепілептичної активності перспективних рослинних антиконвульсантів – сухих екстрактів рутки Шлейхера (СЕРШ) та базилику камфорного (СЕБК). Рослинні препарати вводили внутрішньошлунково в умовно ефективній дозі 100 мг/кг у лікувально-профілактичному режимі протягом трьох днів, востаннє за 30 хв до експерименту. В якості препаратів порівняння було обрано натрію вальпроат та карбамазепін. За умов тесту максимального електрошоку (МЕШ) вивчали здатність обраних сухих екстрактів запобігати розвитку первинно-генералізованих судом. Для поглибленого дослідження протиепілептичних властивостей СЕРШ та СЕБК було обрано модель пентилентетразолового (коразолового) кіндлінгу. Загалом досліджувані сухі екстракти за умов тесту МЕШ виявили виразний протисудомний ефект на рівні натрію вальпроату, проте поступалися за ефективністю карбамазепіну. На моделі пентилентетразолового кіндлінгу було встановлено, що СЕРШ, на відміну від СЕБК та натрію вальпроату, має здатність запобігати розвитку судом за умов експериментального хронічного епілептогенезу. Отже, встановлено, що СЕБК виявляє антиконвульсивну активність на моделі первинно-генералізованих судом, у той час як СЕРШ запобігає розвитку як гострих електростимульованих пароксизмів, так і хронічного епілептогенезу.

ПРОТИВОЭПИЛЕПТИЧЕСКИЙ ПОТЕНЦИАЛ СУХИХ ЭКСТРАКТОВ ДЫМЯНКИ ШЛЕЙХЕРА И БАЗИЛИКА КАМФОРНОГО

В.В.Цивунин, С.Ю.Штрыголь

Ключевые слова: лекарственные растения; экстракты; противозепилептические средства

В работе представлены результаты исследования противозепилептической активности перспективных растительных антиконвульсантов – сухих экстрактов дымянки Шлейхера (СЭДШ) и базилика камфорного (СЭБК). Растительные препараты вводили внутрижелудочно в условно эффективной дозе 100 мг/кг в лечебно-профилактическом режиме в течение трех дней, в последний раз за 30 мин до эксперимента. В качестве препаратов сравнения выбраны натрия вальпроат и карбамазепин. В условиях теста максимального электрошока (МЭШ) изучали способность исследуемых сухих экстрактов предотвращать развитие первично-генерализованных судорог. Для углубленного изучения противозепилептических свойств СЭДШ и СЭБК выбрана модель пентилентетразолового (коразолового) киндлинга. В целом исследуемые сухие экстракты в условиях теста МЭШ выявили значительный противосудорожный эффект на уровне натрия вальпроата, однако уступали по эффективности карбамазепину. На модели пентилентетразолового киндлинга было установлено, что СЭДШ, в отличие от СЭБК и натрия вальпроата, имеет свойство предотвращать развитие судорог в условиях экспериментального хронического эпилептогенеза. Итак, установлено, что СЭБК оказывает антиконвульсивную активность на модели первично-генерализованных судорог, в то время как СЭДШ предотвращает развитие как острых электростимулируемых пароксизмов, так и хронического эпилептогенеза.

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

UDC 615.322: 615.244

THE EFFECT OF THE ANTIDIABETIC COMPOSITION ON THE FUNCTIONAL CONDITION OF THE RAT LIVER IN THE EXPERIMENTAL DIABETES

O.Yu.Koshova

National University of Pharmacy

Key words: diabetes mellitus; hepatopathy; medicinal plants; antidiabetic composition

On the model of diabetes mellitus in rats induced by the 18-days subcutaneous administration of dexamethasone in the dose of 0.125 mg/kg the hepatoprotective properties of a new antidiabetic composition based on medicinal plants have been investigated. The antidiabetic composition and the reference drug – officinal composition “Arphasetinum” were introduced intragastrically as a decoction in the dose of 18 ml/kg simultaneously with dexamethasone. According to the data obtained the antidiabetic composition exhibited a significant hepatoprotective and general metabolic effect. Under the effect of the composition studied the normalization of the basal glycemic level, recovery of the lipid balance and the bile secretory function of the liver, decrease of activity of cytolysis and inflammatory marker enzymes were observed. By its effect on the majority of the indices studied the new antidiabetic composition exceeded the reference composition “Arphasetinum”.

In the last decades a sharp increase in incidence of diabetes mellitus (DM) is observed in most of countries of the world. Almost 90% of cases are accounted for DM type 2. Due to affection of almost all organs and systems, particularly the gastro-intestinal tract, DM becomes a serious medical and social problem [6, 7]. Hyperglycemia, hyperlipidemia, disorders of other links of metabolism are tightly connected with disturbance of the functional activity of the liver – the central organ for metabolism. Numerous clinical trials are the evidence for occurrence of diabetic hepatopathy in patients with DM type 2. In development of the hepatic functional disorders the main role belongs to insulin resistance, which occurs during prediabetes [3]. Taking into account the abovementioned it is reasonable to use drugs, which along with antidiabetic properties have the hepatoprotective activity.

The aim of the work was to investigate the effect of a new antidiabetic composition of medicinal plants on the functional activity of the liver in the experimental diabetes type 2 in rats. The antidiabetic composition contains *Silybum marianum*; *Polygonum aviculare* L.; *Vaccinium vitis-idea* L.; *Linum usitatissimum* L.; *Cichorium intybus* L.; *Zea mays* L.

Materials and Methods

The experiments were conducted on 49 white male rats of old age (18.5 months) with the body mass of 350-400 g. During the experiment animals were kept in standard conditions at 18-24°C, 50-60% of humidity, “day-night” natural light regime, a balanced diet with free access to water. All procedures with animals were conducted according to the principles of the “European convention for the protection of vertebrate animals used for experimental and other scientific purposes” (Strasbourg, 1986), according to the GLP standards [1]. The experimental diabetes was provoked by subcutaneous in-

troductory of dexamethasone in the dose of 0.125 mg/kg for 18 days [4]. The antidiabetic composition was introduced intragastrically as a decoction in the dose of 18 ml/kg simultaneously with dexamethasone once a day [5]. The officinal composition “Arphasetinum” administered in the similar regimen was chosen as a reference drug. The animals were divided into the following groups: intact group (IG); control group (CG); groups of animals that received the antidiabetic composition or a reference composition “Arphasetinum”. On day 19 the bile secretory function of the liver was assessed (the bile secretion rate was calculated within 3 hours in mg/min•100-1g) [2]. Afterwards animals were removed from the experiment by decapitation under chloroform anaesthesia. The level of glucose was determined in the rats' blood. The functional condition of the liver was estimated by biochemical measurements in the blood serum: the activity of such enzymes as alanine aminotransferase (ALT), aspartate transferase (AST), alkaline phosphatase (AF), the cholesterol level, triglycerides (TG), high density lipoproteins (HDL) and low density lipoproteins (LDL) using test kits of “Lachema” company (Czech Republic). The relative mass of liver was determined.

Results and Discussion

Introduction of dexamethasone to old rats (18.5 months) caused a moderate basal hyperglycemia (Table) that was accompanied by expressive lipid disbalance. According to the data obtained the statistically significant increase in the cholesterol level, TG, LDL and decrease in HDL were observed in the blood serum of animals in CG. It indicates development of atherogenic processes under conditions of the experimental diabetes. A relative liver mass increase by 47% compared to IG is the evidence of circulatory dynamics disorder. This disorder occurs as a result of increasing hepatic tissue edema. Increase of alkaline phosphatase in the blood serum almost twice and

Table

A comparative study of the effect of the antidiabetic composition and the composition "Arphasetinum" on the functional condition of the rat liver in dexamethasone diabetes (n = 7)

The indices studied	Groups of animals			
	Intact group	Control group (dexamethasone)	Antidiabetic composition 18 ml/kg + dexamethasone	"Arphasetinum" 18 ml/kg + dexamethasone
Basal glycemia, mmol/l	4.35±0.30	7.15±0.35*	4.38±0.22**	4.03±0.35**
cholesterol, mmol/l	1.47±0.17	2.61±0.25*	1.87±0.15**	1.96±0.15**
LDL, mmol/l	0.38±0.02	0.52±0.02*	0.39±0.04**	0.44±0.05
CL-HDL, mmol/l	2.27±0.28	1.39±0.16*	2.48±0.19**	1.97±0.19†
TG, mmol/l	0.40±0.06	0.63±0.04*	0.43±0.03**/**	0.64±0.05
AP, mmol/l	18.51±6.34	36.35±3.35†	18.97±4.63	17.11±3.90†
ALT, mmol/l·h	0.47±0.01	0.63±0.04*	0.47±0.03**	0.52±0.04**
AST, mmol/l·h	0.42±0.02	0.56±0.04*	0.39±0.03**/**	0.54±0.03*
Bile secretory rate, ml/h·100 ⁻¹	3.56±0.21	2.28±0.29	3.23±0.40	3.03±0.40
Liver relative mass, %	2.30±0.19	3.38±0.21*	2.46±0.20**/†2	3.02±0.16†2

Notes: 1.* – differences statistically significant compared to the intact group, p<0.05; 2.** – differences statistically significant compared to the control group, p<0.05; 3.*** – differences statistically significant compared to the reference drug, p<0.05.

the activity of cytolysis markers of hepatocytes (ALT and AST) in 1.3 times was statistically significant. It is the evidence of development of inflammation in the liver (Table). Destructive consequence in hepatocytes was the bile secretory dysfunction (Table).

Introduction of the antidiabetic composition and the reference drug contributed to normalization of the basal glycemic level. However, compared to the composition "Arphasetinum" under the effect of the antidiabetic composition the more expressive recovery of general trophic processes in the liver and decrease in symptoms of atherogenesis were observed. Dynamics of the relative organ mass and indices of the lipid metabolism was similar to the dynamics in the intact group. The level of cytolytic enzymes and alkaline phosphatase decreased to the physiological level (Table). The bile secretory function

of the liver was restored (Table). Under these conditions the composition "Arphasetinum" exhibited a bit inferior activity. The level of ALT and cholesterol decreased significantly in the group of the reference drug. There was a tendency to normalization of the level of CL-HDL and alkaline phosphatase. However, the content of TG, LDL, AST activity and a relative liver mass were in the same range that in the control group. As a result, the recovery of the bile secretory rate had a character only of an inexpressive tendency (Table).

CONCLUSIONS

Thus, under conditions of the experimental diabetes mellitus induced by dexamethasone it has been found that a new antidiabetic composition exhibits a significant hepatoprotective action due to suppression of inflammation and the positive effect on metabolic processes in the liver.

REFERENCES

1. Директива Совета ЕС о сближении законов, постановлений и администрирование положений государств ЕС по вопросам защиты животных, используемых для экспериментальных и других научных целей (86/609/ЕЕС) / В кн.: Надлежащая производственная практика лекарственных средств. Под ред. Н.А.Ляпунова, В.А.Загория, В.П.Георгиевского, Е.П.Безуглой. – К.: Морион, 1999. – С. 508-545.
2. Дроговоз С.М., Губський Ю.І., Скакун М.П., Коваленко В.М., Деримедвідь Л.В. Експериментальне вивчення жовчогінної, холелітіазної та гепатопротекторної активності нових лікарських засобів: Метод. рекомендації. / За ред. О.В.Стефанова. – К.: Авіценна, 2001. – С. 334-351.
3. Хухліна О.С. // Укр. терапевт. журн. – 2005. – №2. – С. 39-43.
4. Chidambaram Kumarappan Subhash Chandra Mandal // J. Physiol. Pharmacol. – 2014. – Vol. 58, №4. – P. 441-445.
5. Yakovleva L.V., Koshova Y.Y., Laryanovska Y.B. Investigation of properties of "Phytoglunor" [Text] / The 11-th International Congress "Phytopharm 2007", Leiden, The Netherlands, 27-30 June 2007. – Abstract Book, 2007. – P. 163.
6. Velmurugan C., Bhargava A., Kishore Kumar T.S. et al. // JRPNS. – 2013. – Vol. 2, №2. – P. 169-176.
7. Wild S., Roglic G., Green A. et al. // Diabetes Care. – 2004. – Vol. 27. – P. 1047-1053.

ВПЛИВ АНТИДІАБЕТИЧНОГО ЗБОРУ НА ФУНКЦІОНАЛЬНИЙ СТАН ПЕЧІНКИ ЩУРІВ ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ ДІАБЕТІ**О.Ю.Кошова****Ключові слова:** цукровий діабет; гепатопатії; лікарські рослини; антидіабетичний збір

На моделі цукрового діабету у щурів, викликаного 18-денним підшкірним введенням дексаметазону у дозі 0,125 мг/кг, досліджували гепатопротекторні властивості нового збору лікарських рослин, який виявляє антидіабетичні властивості. Антидіабетичний збір та препарат порівняння офіційний збір «Арфазетин» вводили внутрішньошлунково у вигляді відвару (1:10), у дозі 18 мл/кг одночасно з дексаметазоном. Відповідно до отриманих даних антидіабетичний збір виявив виразну гепатопротекторну та загальнометаболичну дію. Під впливом засобу спостерігалась нормалізація рівня базальної глікемії, відновлення ліпідного профілю та жовчосекреторної функції печінки, зниження активності маркерних ферментів цитолізу та запалення. За впливом на більшість досліджуваних показників новий збір переважав препарат порівняння збір «Арфазетин».

ВЛИЯНИЕ АНТИДИАБЕТИЧЕСКОГО СБОРА НА ФУНКЦИОНАЛЬНОЕ СОСТОЯНИЕ ПЕЧЕНИ КРЫС ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ДИАБЕТЕ**Е.Ю.Кошова****Ключевые слова:** сахарный диабет; гепатопатии; лекарственные растения; антидиабетический сбор

На модели сахарного диабета у крыс, вызванного 18-дневным подкожным введением дексаметазона в дозе 0,125 мг/кг исследовали гепатопротекторные свойства нового сбора лекарственных растений с антидиабетическими свойствами. Антидиабетический сбор и препарат сравнения официальный сбор «Арфазетин» вводили внутривентрально в виде отвара (1:10) в дозе 18 мл/кг одновременно с дексаметазоном. Согласно полученным данным, антидиабетический сбор оказывал выраженное гепатопротекторное и общеметаболическое действие. Под воздействием сбора наблюдалась нормализация уровня базальной гликемии, восстановление липидного профиля и желчсекреторной функции печени, снижение активности маркерных ферментов цитолиза и воспаления. По влиянию на большинство изученных показателей новый антидиабетический сбор превосходил препарат сравнения сбор «Арфазетин».

Recommended by Doctor of Medicine, professor T.I. Tiupka

UDC 615.015.13:615.038:616.1

THE STUDY OF THE EFFECT OF THE CHEMOPYRONE SUBSTANCE ON THE ARTERIAL BLOOD PRESSURE UNDER CONDITIONS OF PROPHYLACTIC APPLICATION IN INDUCED HYPERTENSION

N.L.Bereznyakova

National University of Pharmacy

Key words: chemopyrone; diuretic activity; hypertension; systolic and diastolic arterial blood pressure

The most important stage of development of effective and safe new medicines is the methods of preclinical evaluation of their pharmacological activity. Since the existing range of antihypertensive medicines is large enough and the current demands for them are different from those presented several years ago, the aim of this research is to study the effect of the chemopyrone substance on the arterial pressure under conditions of prophylactic application on the experimental model of hypertension induced by Mesatonum. While studying the effect of the chemopyrone substance in the dose of 10 mg/kg on the level of systolic and diastolic arterial pressure it has been determined that the antihypertensive effect is achieved due to the impact on the function of the kidneys. The maximum effect is observed on the 10-th min and equals 64-62% compared to the group of intact animals. The results obtained testify the expressed and prolonged hypotensive effect, and the substance can be recommended for hypertensive states correction in the conditions of prophylactic application.

Increase of effectiveness of the arterial hypertension treatment is still a topical problem of medicine and pharmacy because the causes of this disease, in spite of intensive research in this area, are still unknown [5].

Based on medical statistics data the role of some factors ("risk factors"), such as heredity, age, sex, nutritional peculiarities, weight and others, which can influence on the arterial hypertension development was determined [2].

The results of the study not only demonstrate dependence of cardiovascular pathology development on the arterial pressure level (AT), but also allow to evaluate quantitatively the contribution of the systolic (ATS) and diastolic (ATD) arterial pressure to the risks degree [10]. Usually, ATS increases continually with age and is a strong, independent, but variable index of cardiovascular complications of hypertension. Underestimation of its value leads to underestimation of the prevalence of hypertension and untimely prescription of antihypertensive medicines.

Most studies indicate the promising use of rational combinations of diuretics and β -adrenoreceptor antagonists (in low doses) that provide the additive effect, as well as the use of drugs with multiple mechanisms of activity to reach the target level of AT.

As the alternative in regards to the mentioned above the medicines based on pyrol[3,2,1-*ij*]quinoline may be applied for the treatment of hypertension [11]. They do not lead to water and electrolyte imbalance and do not cause the secondary hypertension, as well as exhibit the diuretic activity.

At the Pharmaceutical Chemistry Department of the NUPh 4-methoxyanilide of 1-hydroxy-3-oxy-5,6-dihydro-3*H*-pyrol[3,2,1-*ij*]quinoline-2-carbolic acids under

the conditional name of chemopyrone was synthesized. This compound is practically nontoxic and exhibits the expressed diuretic activity [12].

The aim of the present study was to study the effect of the chemopyrone substance on the arterial pressure under conditions of prophylactic application on the experimental model of induced hypertension.

Materials and Methods

The research was conducted on nonlinear white male rats weighting 180-230 g. The effect of the chemopyrone substance on the arterial pressure was studied both on intact animals and on the background of Mesatonum-induced hypertension.

Mesatonum stimulates postsynaptic α_1 -adrenoreceptor antagonists and increases percussive emission, thereby, rises systolic and diastolic AT by decreasing the pulse reflectively [3]. Mesatonum was injected intraperitoneally in the dose of 1 mk/kg [8, 9]. The observation time of animals after Mesatonum injection lasted for 10 minutes.

In total, three experimental groups were formed (each group consisted of 6 animals).

Group 1 – the intact animals injected with Mesatonum only.

Group 2 – the positive control (a single intragastrical dose of peryndopril + Mesatonum in 1 hour).

"Prestarium" tablets (the active substance – 4 mg of perindopril) produced by "Servier's Laboratory" company, France was used as a reference drug. The total mass of a tablet is 90 mg. Dosing was carried out based on the active component. Since the maximum therapeutic dose for human equals 0.15 mg/kg, the extrapolated dose of 1.5 mg/kg was calculated for rats.

Group 3 – the chemopyrone substance in the dose of 10 mg/kg + Mesatonum in 1 hour.

Table 1

Characteristics of Mesatonum-induced hypertension in the control group

No.	AP MmHg before Mesatonum injection Systolic / Diastolic		AP MmHg + Mesatonum on the 10-th min Systolic / Diastolic	
1	134	83	150	91
2	122	76	145	80
3	127	70	132	75
4	136	83	140	90
5	119	78	125	80
6	130	71	136	75
M	128.00	76.83	138	81.83
m	2.72	2.30	6.30	1.24

Table 2

Characteristics of Mesatonum-induced hypertension in the group with the chemopyrone substance

No.	AP MmHg before Mesatonum injection Systolic / Diastolic		AP MmHg + Mesatonum on the 10-th min Systolic / Diastolic	
1	137	86	142	90
2	135	75	145	82
3	123	65	130	63
4	142	94	140	96
5	124	82	134	79
6	124	59	129	63
M	130.83	76.83	136.67	78.83
m	3.34	5.38	4.59	3.98

During the research with the help of a RM-6000 polygraph (Japan) the ATS and ATD indices of non-anesthetized animals were registered. The rats were placed into special cages-cases of a transparent plastic. The cuff and transducer were put on the tail. Automatically, the pressure was given into the cuff, and the value of the external pressure was determined when the transducer stopped registering the pulse vibrations [1].

The comparative effectiveness in the groups was estimated according to several indices [4, 6, 7, 8]:

- Systolic arterial pressure.
- Diastolic arterial pressure.

The hypotensive activity was examined when reducing the arterial pressure by 25 MmHg and more.

Experiments on animals were conducted according to the Council Directive 2010/63/EU of 22 September 2010 on the protection of animals used for scientific purposes.

The results of the studies were processed in accordance with the Van der Waerden's criterion (SW package Statistica 6).

Results and Discussion

Introduction of Mesatonum to the group of intact animals accompanied with a rapid development of hypertension – in all cases the increase of systolic (by an average of 80 MmHg) and diastolic (by an average of 40 MmHg) arterial pressure was observed. At the same time it was determined that the level of ATS was $212 \pm$

± 8.5 MmHg, and ATD 125 ± 7.6 MmHg. The effect reached the maximum on the 10-th min of observation and equaled 60–47% of the baseline values.

Prophylactic introduction of perindopril in the group of animals with Mesatonum-induced hypertension within the time of observation prevented the arterial pressure rising and equaled: ATS 138 ± 6.3 MmHg, ATD 81.83 ± 1.24 MmHg (Table 1).

Injection of chemopyrone in the dose of 10 mg/kg on the model of Mesatonum-induced hypertension presumably prevents increasing arterial pressure. The maximum effect is observed on the 10-th min and equals 64–62% compared to the group of intact animals.

As the data presented in Table 2 testify with the prophylactic use of the chemopyrone substance no significant increase of systolic and diastolic pressure was observed. It indirectly reflects a pronounced and prolonged hypotensive effect in this dose. Significant distinctions compared to perindopril were not observed as well.

CONCLUSIONS

1. On the model of Mesatonum-induced hypertension the chemopyrone substance has revealed the expressed hypotensive activity, considerably reducing the level of systolic and diastolic arterial pressure.

2. The data obtained allow to consider the chemopyrone substance as a promising chemical compound for creating a medicine on its basis for hypertensive states correction under conditions of prophylactic application.

REFERENCES

1. Біохімічний склад рідин організму та їх клініко-діагностичне значення / За ред. О.Я.Солярова. – К.: Здоров'я, 2004. – 192 с.
2. Кобалава Ж.Д., Моисеев В.С. // Клин. фармакол. и терапия. – 2004. – №13. – С. 10-18.
3. Машковский М.Д. Лекарственные средства. – 15-е изд. – М.: РИА «Новая волна»; Издатель Умеренков, 2009. – С. 873-897.
4. Меньшиков В.В. Методики клинических лабораторных исследований. – М.: Лабора, 2009. – 880 с.
5. Моисеев В.С., Кобалава Ж.Д. // Клин. фармакол. и терапия. – 2000. – №5. – С. 86-96.
6. Назаренко Г.И., Кишкун А.А. Клиническая оценка результатов лабораторных исследований. – М.: Медицина, 2000. – 544 с.
7. Прозоровский В.Б. // Психофармакол. и биол. наркол. – 2007. – Т. 7, №3-4. – С. 2090-2120.

8. Хабриев Р.У. Руководство по экспериментальному (доклиническому) изучению новых фармакологических веществ / Под общ. ред. чл.-кор. РАМН, проф. Р.У.Хабриева. – 2-е изд. – М.: ОАО Изд-во «Медицина», 2005. – 832 с.
9. Харкевич Д.А. Фармакология. – 10-е изд. – М.: ГЭОТАР-Медиа, 2010. – 908 с.
10. Шальнова С.А., Деев А.Д., Оганов Р.Г., Шестов Д.Б. // Кардиоваскулярная терапия и профилактика. – 2002. – №1. – С. 10-15.
11. Ukrainets I.V., Berezhnyakova N.L., Grinevich L.A. et al. // Chem. Heterocycl. Comp. – 2010. – Vol. 46, №6. – P. 699-710.
12. Ukrainets I.V., Mospanova E.V., Berezhnyakova N.L., Naboka O.I. // Chem. Heterocycl. Comp. – 2007. – Vol. 43, №12. – P. 1532-1539.

ДОСЛІДЖЕННЯ ВПЛИВУ СУБСТАНЦІЇ ХІМОПІРОН НА АРТЕРІАЛЬНИЙ ТИСК В УМОВАХ ПРОФІЛАКТИЧНОГО ЗАСТОСУВАННЯ ПРИ ІНДУКОВАНІЙ ГІПЕРТЕНЗІЇ

Н.Л.Березнякова

Ключові слова: хімопірон; діуретична активність; гіпертензія; систолічний і діастолічний артеріальний тиск

Найважливішим етапом розробки ефективних та безпечних нових лікарських засобів є методи доклінічної оцінки їх фармакологічної активності. Оскільки існуючий арсенал антигіпертензивних засобів великий і сучасні вимоги, які висувуються до них, відрізняються від тих, що мали місце кілька років тому, метою даної роботи стало вивчення впливу субстанції хімопірон на артеріальний тиск в умовах профілактичного застосування на експериментальній моделі індукованої мезатоном гіпертензії. При дослідженні впливу субстанції хімопірон у дозі 10 мг/кг на рівень систолічного та діастолічного артеріального тиску встановлено, що антигіпертензивний ефект досягається за рахунок впливу на діяльність нирок. Максимальний ефект спостерігається на 10-й хв та становить 64-62% у порівнянні з групою інтактних тварин. Одержані результати свідчать про виражений та тривалий гіпотензивний ефект, а субстанція може бути рекомендована для корекції гіпертензивних станів в умовах профілактичного застосування.

ИССЛЕДОВАНИЕ ВЛИЯНИЯ СУБСТАНЦИИ ХИМОПИРОН НА АРТЕРИАЛЬНОЕ ДАВЛЕНИЕ В УСЛОВИЯХ ПРОФИЛАКТИЧЕСКОГО ПРИМЕНЕНИЯ ПРИ ИНДУЦИРОВАННОЙ ГИПЕРТЕНЗИИ

Н.Л.Березнякова

Ключевые слова: хімопірон; диуретическая активность; гипертензия; систолическое и диастолическое артериальное давление

Важнейшим этапом разработки эффективных и безопасных новых лекарственных средств являются методы доклинической оценки их фармакологической активности. Поскольку существующий арсенал антигипертензивных средств большой и современные требования, предъявляемые к ним, отличаются от тех, что имели место несколько лет назад, целью данной работы стало изучение влияния субстанции хімопірон на артериальное давление в условиях профилактического применения на экспериментальной модели индуцированной мезатоном гипертонии. При исследовании влияния субстанции хімопірон в дозе 10 мг/кг на уровень систолического и диастолического артериального давления установлено, что антигипертензивный эффект достигается за счет воздействия на деятельность почек. Максимальный эффект наблюдается к 10-й мин и составляет 64-62% по сравнению с группой интактных животных. Полученные результаты свидетельствуют о выраженном и длительном гипотензивном эффекте, а субстанция хімопірон может быть рекомендована для коррекции гипертензивных состояний в условиях профилактического применения.

Recommended by Doctor of Pharmacy, professor K.G.Schokina

UDC 615.322:615.275.4

DETERMINATION OF THE ELGACIN EFFECT ON THE CELLULAR COMPONENT OF THE IMMUNE SYSTEM IN AGED RATS

O.Yu.Koshova

National University of Pharmacy

Key words: ageing; cell-mediated immunity; geroprotectors; ellagotanins, elgacin

The study of the effect of a new original drug with antioxidant properties – “Elgacin” tablets on cellular component of the immune system of old mice in conditions of the delayed hypersensitivity reaction has been conducted. It has been found that decrease in reactivity of the cell-mediated response takes place in ageing. Introduction of “Elgacin” tablets to old mice contributed to restoration of the immune response on introduction of the antigen up to the physiological level of young animals. It testify in favour of its potential geroprotective properties regarding age-dependent changes of the immunity. The data obtained substantiate the further research of “Elgacin” tablets.

Gradual immunity suppression takes place during the ageing process. It results in increase of frequency and severity of infectious diseases, cancer and autoimmune disorders. First of all, during ageing the functionality of the T-system of the immunity changes, in particular the ability to distinguish the allotypic antigen by macrophagocytes and lymphocytes. The activity of helper T-cells (not only TH1, but also TH2) is suppressed, the suppressor function of the immune system is disturbed, the activity of metabolic processes in phagocytes and other cells that are responsible for nonspecific anti-infectious reactivity is decreased [6].

Thus, typical diseases of elderly age are associated with decrease of the immune reactivity as a result of profound changes in the population structure of T-cells and their functions. It is also the result of the activity of cells that participate in nonspecific cell-mediated and antibody-mediated reactions in ageing.

The abovementioned determines the relevance of search for a geroprotective agent with affinity to the immune system. The Central Research Laboratory of the National University of Pharmacy conducts the study of a new original antioxidant drug “Elgacin” as a geroprotector. The active substances of “Elgacin” are ellagotanins isolated from collective fruit of sticky adler and grey adler (*Alnus glutinosa* L., *Alnus cinerea* L.). In experiments on rats of different age it has been found that administration of “Elgacin” prevents development of age dependent disorders of the liver, heart, carbohydrate and lipid metabolism [2].

The aim of this work was to determine the effect of “Elgacin” tablets on the cellular component of the immune system of aged rats.

Materials and Methods

The experiments were conducted on 100 outbred male mice of the young fertile age (6 months with the body mass of 18.0-20.0 g) and mice of the old age (20 months, 30-35 g). During the experiment animals were kept in

standard conditions at 18-24°C, 50-60% of humidity, “day-night” natural light regime, a balanced diet with free access to water. All procedures with animals were performed according to the principles of the “European convention for the protection of vertebrate animals used for experimental and other scientific purposes” (Strasbourg, 1986), according to the GLP standards [1]. The condition of the cell mediated immunity was assessed by the delayed hypersensitivity reaction using the method of K.P.Kitamura with determination of the reaction index (RI) [5]. Mice were immunized by thymus-dependent antigen – sheep erythrocytes (SE). The animals were divided into the following groups (10 animal in each group): group 1-2 – nonimmune (intact) control of the fertile age and old age; group 3-4 – immunized control (SE) of the fertile and old age; group 5-6 – animals of the old age that received the drug under research in the dose of 1.4 and 14 mg/kg before and during the whole period of immunization by SE. Doses of “Elgacin” tablets for mice were re-calculated from a conditionally therapeutic dose for rats previously determined taking into account the dose conversion factor by body area [3]. The drug studied was introduced to mice intragastrically once a day within 3 days prior and during the whole immunization period.

The experimental data obtained were processed using methods of analysis of variance with the help of the “Statistica 6.0” statistic programme.

Results and Discussion

As it was mentioned above, the progressive suppression of all components of the immune system occurs while ageing. The highest immune response is registered during the period of puberty, but in elderly people it composes only 1-2% from this level. Progressive suppression of the thymus-dependent immune component is associated with aged-related involution of the thymus. This is expressed in its mass reduction, weakening of its function and synthesis of regulatory factors. The humoral compo-

Reaction index of delayed hypersensitivity in mice of different age

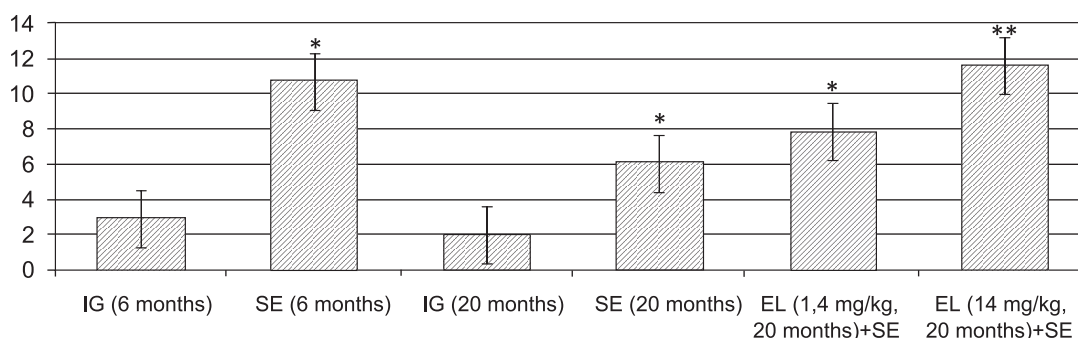


Fig. The effect of "Elgacin" tablets in the doses of 1.4 and 14 mg/kg on the cell-mediated immune response of mice with the normal immune status (n = 10).

Notes: IG (6 months); IG (20 months) – the intact control of fertile and old age, respectively; SE (6 months); SE (20 months) – the immunized control of the fertile and old age, respectively. EL (1.4; 14 mg/kg, 20 months)+SE – mice aged 20 months that received "Elgacin" in the doses of 1.4 and 14 mg/kg on the background of immunization.

* – statistically significant differences compared to the intact group of the fertile age, $p < 0.05$;

** – statistically significant differences compared to the immunized SE group of the old age, $p < 0.05$.

nent of the immunity also undergoes negative changes in ageing: decrease in the level of normal antibodies, including isohaemoglutinins, is observed [4].

According to the data obtained there was the normal immune response to introduction of sheep erythrocytes in young mice (Fig.). In old mice the decrease in the reaction index in 1.6 times compared to immunized animals of the fertile age was registered. It indicates a significant decrease in the immune response on introduction of the thymus-dependent antigen (Fig.).

When introducing "Elgacin" tablets in the dose of 1.4 mg/kg the tendency to increase the reaction index of delayed hypersensitivity was observed (Fig.). The increase in the dose of the tablets led to restoration of the immune response in old mice up to the normal level. The RI of delayed hypersensitivity in mice that received the drug studied was almost twice higher compared to old animals. And it had no difference from the values of

the RI in the group of immunized animals of the fertile age (Fig.). The given differences between groups were statistically significant.

A positive effect of "Elgacin" tablets on the immune system of aged rats apparently can be explained by stimulating impact of elagotanins on inflammation mediators of the cellular component of the immunity determined in the experiments *in vivo* and *in vitro*, in particular interleukin 1 and tumour necrosis factor alpha ((TNF- α) [7, 8].

CONCLUSIONS

1. The results of the study conducted confirm the literature data concerning decrease of the immune reactivity in ageing.

2. The data obtained demonstrate the immunomodulatory properties of "Elgacin" tablets towards the cellular component of the immunity in old mice. These data substantiate the further research in this field.

REFERENCES

1. Директива Совета ЕС о сближении законов, постановлений и администрирование положений государств ЕС по вопросам защиты животных, используемых для экспериментальных и других научных целей (86/609/ЕЕС) / В кн.: Надлежащая производственная практика лекарственных средств; Под ред. Н.А.Ляпунова, В.А.Загория, В.П.Георгиевского, Е.П.Безуглой. – К.: Морион, 1999. – С. 508-545.
2. Яковлева Л.В., Карбушева И.В., Лар'яновська Ю.Б. // Фармаком. – 2004. – №3. – С. 36-41.
3. Freireich E.J., Gehan E.A., Ral D.P. et al. // Cancer Chemother. Rep. – 1966. – Vol. 50, №4. – P. 219-244.
4. Hajo Haase. The immune system and the impact of zinc during aging / Hajo Haase, Lothar Rink [Electronic resource] // Immunity & Ageing. – 2009. – №6 (9). – Access mode: <http://www.immunityageing.com/content/6/1/9>.
5. Kitamura K.A. // J. Immunol. Methods. – 1980. – Vol. 39. – P. 277-283.
6. Meyer K.C. // Proc. Am. Thorac. Soc. – 2005. – №2. – P. 433-439.
7. Miyamoto K., Murayama T., Nomura M. et al. // Anticancer Res. – 1993. – Vol. 13. – P. 37-42.
8. Yoshida T., Amakura Y., Yoshimura M. // Int. J. Mol. Sci. – 2010. – Vol. 6; №11 (1). – P. 79-106.

ВИЗНАЧЕННЯ ВПЛИВУ ЕЛГАЦИНУ НА КЛІТИННУ ЛАНКУ ІМУНІТЕТУ СТАРИХ МИШЕЙ**О.Ю.Кошова****Ключові слова:** старіння; клітинний імунітет; геропротектори; елаготаніни; елгацин

Проведено вивчення впливу нового оригінального засобу з антиоксидантними властивостями таблеток «Елгацин» на клітинну ланку імунітету старих мишей за умов реакції гіперчутливості повільного типу. Встановлено, що при старінні спостерігається зниження реактивності клітинного імунітету. Введення таблеток «Елгацин» старим мишам сприяло відновленню імунної відповіді на введення антигену до фізіологічного рівня молодих тварин, що свідчить про його потенційні геропротекторні властивості щодо вікових змін імунітету. Отримані дані обґрунтовують перспективність подальших досліджень елгацину у цьому напрямку.

ОПРЕДЕЛЕНИЕ ВЛИЯНИЯ ЭЛГАЦИНА НА КЛЕТОЧНОЕ ЗВЕНО ИММУНИТЕТА СТАРЫХ МЫШЕЙ**Е.Ю.Кошова****Ключевые слова:** старение; клеточный иммунитет; геропротекторы; элаготанины; элгацин

Проведено изучение влияния нового оригинального препарата с антиоксидантными свойствами таблеток «Элгацин» на клеточное звено иммунитета старых мышей в условиях реакции гиперчувствительности замедленного типа. Установлено, что при старении наблюдается снижение реактивности клеточного иммунитета. Введение таблеток «Элгацин» старым мышам способствовало восстановлению иммунного ответа на введение антигена до физиологического уровня молодых животных, что свидетельствует о его потенциальных геропротекторных свойствах относительно возрастных изменений иммунитета. Полученные данные обосновывают перспективность дальнейших исследований элгацина в этом направлении.

ЗМІСТ / CONTENTS / СОДЕРЖАНИЕ

ЮВІЛЕЙ В.П.ЧЕРНИХ	3
-------------------------	---

СИНТЕЗ ТА АНАЛІЗ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН

SYNTHESIS AND THE STUDY OF THE ANTIMICROBIAL ACTIVITY OF 3-AMINO-5-METHYL-2-(ALKYLTHIO)-4-OXO-N-ARYL-3,4-DIHYDROTHIENO[2,3- <i>d</i>]PYRIMIDINE-6-CARBOXAMIDES / S.V.Vlasov, S.M.Kovalenko, T.P.Osodolchenko, V.P.Chernykh	6
Синтез та дослідження антимікробної активності 3-аміно-5-метил-2-(алкілтіо)-4-оксо- <i>N</i> -арил-3,4-дигідротієно[2,3- <i>d</i>]піримідин-6-карбоксамідів / С.В.Власов, С.М.Коваленко, Т.П.Осолодченко, В.П.Черних	
Синтез и исследование противомикробной активности 3-амино-5-метил-2-(алкилтио)-4-оксо- <i>N</i> -арил-3,4-дигидротиено[2,3- <i>d</i>]пиримидин-6-карбоксаминов / С.В.Власов, С.Н.Коваленко, Т.П.Осолодченко, В.П.Черных	
SYNTHESIS AND MODIFICATION OF 2-[(8- <i>R</i> ₁ -9- <i>R</i> ₂ -10- <i>R</i> ₃ -3- <i>R</i> -2-OXO-2 <i>H</i> -[1,2,4]TRIAZINO[2,3- <i>c</i>]QUINAZOLINE-6-YL)THIO]ACETIC ACIDS AIMED AT SEARCHING EFFECTIVE SUBSTANCES WITH THE ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY / I.S.Nosulenko, O.Yu.Voskoboinik, G.G.Berest, S.I.Kovalenko, O.M.Kamyshnyi, N.M.Polishchuk	11
Синтез та модифікація 2-[(8- <i>R</i> ₁ -9- <i>R</i> ₂ -10- <i>R</i> ₃ -3- <i>R</i> -2-оксо-2 <i>H</i> -[1,2,4]триазино[2,3- <i>c</i>]хіназолін-6-іл)тіо]оцтових кислот, спрямовані на пошук сполук з антибактеріальною та протигрибковою дією / І.С.Носуленко, О.Ю.Воскобойнік, Г.Г.Берест, С.І.Коваленко, О.М.Камішний, Н.М.Поліщук	
Синтез и модификация 2-[(8- <i>R</i> ₁ -9- <i>R</i> ₂ -10- <i>R</i> ₃ -3- <i>R</i> -2-оксо-2 <i>H</i> -[1,2,4]триазино[2,3- <i>c</i>]хиназолин-6-ил)тио]уксусных кислот, направленные на поиск соединений с антибактериальным и противогрибковым действием / И.С.Носуленко, А.Ю.Воскобойник, Г.Г.Берест, С.И.Коваленко, А.М.Камышный, Н.М.Полищук	
VOLTAMMETRIC DETERMINATION OF CEFOTAXIME USING POTASSIUM PEROXOMONOSULFATE / Yu.Yu.Labuzova	21
Вольтамперометричне визначення цефотаксиму за участю калію гідрогенопероксомоносульфату / Ю.Ю.Лабузова	
Вольтамперометрическое определение цефотаксима с участием калия гидропероксомоносульфата / Ю.Ю.Лабузова	
VERIFICATION OF HPLC FOR THE QUANTITATIVE DETERMINATION METHOD OF NIFEDIPINE IN TABLETS / I.L.Komarytsky, V.A.Khanin, N.Yu.Bevz, V.A.Georgiyants	25
Верифікація ВЕРХ методики кількісного визначення ніфедипіну в таблетках / І.Л.Комарицький, В.А.Ханін, Н.Ю.Бевз, В.А.Георгіянц	
Верификация ВЭЖХ методики количественного определения нифедипина в таблетках / И.Л.Комарицкий, В.А.Ханин, Н.Ю.Бевз, В.А.Георгиянц	
THE ASSESSMENT OF THE METHOD FOR QUANTITATIVE DETERMINATION OF PREDNISOLONE IN THE OINTMENT BY THE REACTION WITH PHENYLHYDRAZINE / O.A.Ievtifieieva, K.I.Proskurina, O.M.Ganieva, V.T.Kirdan	30
Оцінка методики кількісного визначення преднізолону в мазі за реакцією з фенілгідразином / О.А.Євтіфєєва, К.І.Проскуріна, О.М.Ганєва, В.Т.Кірдан	
Оценка методики количественного определения преднизолона в мази по реакции с фенилгидразином / О.А.Евтифеева, К.И.Проскурина, О.М.Ганева, В.Т.Кирдан	
THE STUDY OF THE ELEMENTAL COMPOSITION OF SUMMER SQUASH (<i>CUCURBITA PEPO L.</i>) / Yu.A.Fedchenkova, I.I.Batyuchenko, O.P.Khvorost	34
Дослідження елементного складу сировини гарбуза звичайного <i>Cucurbita pepo L.</i> / Ю.А.Федченкова, І.І.Батюченко, О.П.Хворост	
Исследование элементного состава сырья тыквы обыкновенной <i>Cucurbita pepo L.</i> / Ю.А.Федченкова, И.И.Батюченко, О.П.Хворост	

ТЕХНОЛОГІЯ ЛІКАРСЬКИХ ПРЕПАРАТІВ

THE STUDY OF THE PROPERTIES OF EMULSIONS BASED ON SEPIPLUS 400 / T.Kovalyova, N.Polovko	38
Вивчення властивостей емульсій на основі Сепіплус 400 / Т.М.Ковальова, Н.П.Половко	
Изучение свойств эмульсий на основе Сепиплюс 400 / Т.Н.Ковалева, Н.П.Половко	
SUBSTANTIATION FOR SELECTING A PRESERVATIVE WHEN DEVELOPING THE GEL WITH ESSENTIAL OILS FOR TREATING DISEASES OF THE UPPER RESPIRATORY TRACT / V.V.Pul-Luzan, O.P.Strilets, T.V.Martynuk	42
Обґрунтування вибору консерванта при розробці гелю з ефірними оліями для лікування захворювань верхніх дихальних шляхів / В.В.Пуль-Лузан, О.П.Стрілець, Т.В.Мартинюк	
Обоснование выбора консерванта при разработке геля с эфирными маслами для лечения заболеваний верхних дыхательных путей / В.В.Пуль-Лузан, О.П.Стрелец, Т.В.Мартынюк	
DEVELOPMENT OF THE SHAMPOO FOR CHILDREN / L.S.Petrovskaya, O.V.Zhuk, I.I.Baranova	45
Розробка складу шампуню для дітей / Л.С.Петровська, О.В.Жук, І.І.Баранова	
Разработка состава шампуня для детей / Л.С.Петровская, Е.В.Жук, И.И.Баранова	

ОРГАНІЗАЦІЯ ТА ЕКОНОМІКА ФАРМАЦІЇ

ANALYSIS OF THE ASSORTMENT OF IMMUNOBIOLOGICAL MEDICAL PRODUCTS USED FOR CHILDREN ROUTINE IMMUNIZATION AT THE UKRAINIAN PHARMACEUTICAL MARKET / A.A.Kotvitska, O.V.Kononenko	49
Аналіз асортименту медичних імунобіологічних препаратів, що використовуються для планової вакцинопрофілактики дітей на фармацевтичному ринку України / А.А.Котвіцька, О.В.Кононенко	
Анализ ассортимента медицинских иммунобиологических препаратов, которые используются для плановой вакцинопрофилактики детей на фармацевтическом рынке Украины / А.А.Котвицкая, О.В.Кононенко	
ANALYSIS OF THE ATTITUDE OF PHARMACY SPECIALISTS TOWARDS THE CURRENT SOCIAL PROTECTION SYSTEM AND DIRECTIONS FOR ITS REFORMATION / M.V.Zarichkova	55
Аналіз ставлення спеціалістів фармації до існуючої системи соціального захисту та напрямки її реформування / М.В.Зарічкова	
Анализ отношения специалистов фармации к существующей системе социальной защиты и пути ее реформирования / М.В.Заричкова	
THE STUDY OF EXTEMPORANEOUS COMPOUNDING OF HOMEOPATHIC MEDICINAL PRODUCTS / V.M.Tolochko, D.V.Vakulenko, I.V.Shyshkina	60
Вивчення екстемпоральної рецептури гомеопатичних лікарських засобів / В.М.Толочко, Д.В.Вакулєнко, І.В.Шишкіна	
Изучение экстенпоральной рецептуры гомеопатических лекарственных средств / В.М.Толочко, Д.В.Вакулєнко, И.В.Шишкина	

ЕКСПЕРИМЕНТАЛЬНА ТА КЛІНІЧНА ФАРМАКОЛОГІЯ

ANTIPILEPTIC POTENTIAL OF <i>FUMARIA SCHLEICHERI</i> AND <i>OCIMUM BASILICUM</i> DRY EXTRACTS / V.V.Tsyvulin, S.Yu.Shtyrol'	64
Протиепілептичний потенціал сухих екстрактів рутки шлейхера та базилику камфорного / В.В.Цивунін, С.Ю.Штриголь	
Противоэпилептический потенциал сухих экстрактов дымянки шлейхера и базилика камфорного / В.В.Цывунин, С.Ю.Штрыголь	
THE EFFECT OF THE ANTIDIABETIC COMPOSITION ON THE FUNCTIONAL CONDITION OF THE RAT LIVER IN THE EXPERIMENTAL DIABETES / O.Yu.Koshova	69
Вплив антидіабетичного збору на функціональний стан печінки щурів при експериментальному діабеті / О.Ю.Кошова	
Влияние антидиабетического сбора на функциональное состояние печени крыс при экспериментальном диабете / Е.Ю.Кошова	
THE STUDY OF THE EFFECT OF THE CHEMOPYRONE SUBSTANCE ON THE ARTERIAL BLOOD PRESSURE UNDER CONDITIONS OF PROPHYLACTIC APPLICATION IN INDUCED HYPERTENSION / N.L.Bereznaykova	72
Дослідження впливу субстанції хімопірон на артеріальний тиск в умовах профілактичного застосування при індукованій гіпертензії / Н.Л.Березнякова	
Исследование влияния субстанции химопирон на артериальное давление в условиях профилактического применения при индуцированной гипертензии / Н.Л.Березнякова	
DETERMINATION OF THE ELGACIN EFFECT ON THE CELLULAR COMPONENT OF THE IMMUNE SYSTEM IN AGED RATS / O.Yu.Koshova	75
Визначення впливу елгацину на клітинну ланку імунітету старих мишей / О.Ю.Кошова	
Определение влияния элгацина на клеточное звено иммунитета старых мышей / Е.Ю.Кошова	
Авторський показник статей журналу "Вісник фармації" за 2014 рік	78

Адреса для листування: 61002, м. Харків, вул. Пушкінська, 53, Національний фармацевтичний університет, редакція журналу "Вісник фармації", тел./факс (0572) 68-00-24. E-mail: press@ukrfa.kharkov.ua.
Передплатні індекси: для індивідуальних передплатників — 74102; для підприємств — 74103.

Свідectво про державну реєстрацію серія KB №14938-3910ПР від 04.02.2009 р.

Підписано до друку 16.03.2015 р. Формат 60x84 1/8. Папір офсетний. Друк ризографія.
Умовн. друк. арк. 10,23. Обліков.-вид. арк. 11,87. Тираж 100 прим.

Літературні редактори О.Ю.Гурко, А.Л.Краснікова; комп'ютерна верстка О.М.Білінська.