МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

Рік заснування – 1993

Назустріч VIII Національному з'їзду фармацевтів України

ВІСНИК ФАРМАЦІЇ

NEWS OF PHARMACY

ВЕСТНИК ФАРМАЦИИ

2016 – №1 (85)

Харків
НФаУ

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

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

Рекомендовано Вченою радою Національного фармацевтичного університету
(протокол №6 від 26.02.2016 р.)

Журнал "Вісник фармації" включений до затвердженого МОН України Переліку наукових фахових видань України для опублікування результатів дисертаційних робіт з медичних та фармацевтичних наук (наказ МОН України від 06.03.2015 р. №261).

З 2002 року Chemical Abstracts Service здійснює відбір та розміщення електронних версій рефератів журналу "Вісник фармації" на своїй веб-сторінці: http://www.cas.org (код журналу: VFIAA2)

Назустріч VIII Національному з'їзду фармацевтів України

Вельмишановні колеги!

Одним із головних завдань фармацевтичного сектора галузі охорони здоров'я є забезпечення населення якісними, ефективними і доступними ліками, надання фармацевтичної допомоги, збереження потенціалу здоров'я та працездатності українського народу.

З'їзди фармацевтів, на яких фармацевтичне співтовариство обговорювало нагальні проблеми і напрямки подальшого розвитку сектора галузі охорони здоров'я, завжди були значущими і необхідними.

За часів незалежності нашої держави стало доброю традицією проводити професійне зібрання найвищого рівня, яке об'єднує всі складові фармації, у Харкові — «фармацевтичній столиці» України на базі Національного фармацевтичного університету.

Кожен з'їзд фармацевтів — це значуща історична подія, можливість не тільки вшанувати чотирьохсоттисячне фармацевтичне співтовариство, але й підбити підсумки та обмінятися досвідом із зарубіжними колегами, представити стратегію подальшого розвитку.

V З'їзд фармацевтів України вперше серед країн СНД заснував професійне свято — День фармацевтичного працівника України. Минуло понад 10 років, і Міжнародна фармацевтична федерація запропонувала щорічно 25 вересня святкувати Всесвітній день фармацевта. V З'їзд фармацевтів України отримав статус Національного, і саме з 1999 р. ведуть свою історію галузеві форуми найвищого рівня.

На VI Національному з'їзді була прийнята Концепція розвитку фармацевтичної галузі України та впроваджене почесне звання «Заслужений працівник фармації України», а на VII був презентований результат спільної кількарічної праці — Етичний кодекс фармацевтичних працівників України. Крім того, оргкомітет представив друге видання «Фармацевтичної енциклопедії» та довідник підприємств і установ фармацевтичного сектора «Фармація України».

Згідно з посвідченням № 113 від 21 квітня 2015 р., виданим Українським інститутом науково-технічної та економічної інформації, на базі Національного фармацевтичного університету 13-16 вересня 2016 р. відбудеться VIII Національний з'їзд фармацевтів України.

За одноголосним рішенням делегатів VII Національного з'їзду фармацевтів України черговий форум відбудеться у Харкові на базі Національного фармацевтичного університету.

За славною традицією з'їзд збереже формат свого проведення та пройде як діалог між представниками усіх сегментів галузі — освіти, науки, виробництва, дистрибуції, контролю якості, аптечної мережі, інформаційного поля, громадських організацій. У рамках з'їзду будуть висвітлені досягнення фармації, визначені вектори розвитку та обговорені нагальні проблеми. Серед них особливо гостро стоять проблеми підвищення ролі фармацевта в сучасній системі охорони здоров'я та розробка і запровадження нової концепції підготовки фармацевтичних кадрів, об'єднання численних професійних асоціацій (з імплементацією зарубіжних підходів) в єдину Всеукраїнську громадську організацію.

З огляду на коло питань, винесених до розгляду делегатів VIII Національного з'їзду фармацевтів України, галузевий форум, безсумнівно, стане видатною подією не тільки для вітчизняної фармації, а й для держави в цілому.

З повагою,
В.П. Черних,
ректор Національного фармацевтичного університету, академік НАН України, доктор фармацевтичних наук, доктор хімічних наук, заслужений діяч науки і техніки УРСР, заслужений винахідник УРСР, лауреат Державної премії України, професор.

VIII НАЦІОНАЛЬНИЙ З'ЇЗД ФАРМАЦЕВТІВ УКРАЇНИ
13-16 вересня 2016 року, м. Харків

ІНФОРМАЦІЙНЕ ПОВІДОМЛЕННЯ № 1

Організатори з'їзду:
- Міністерство охорони здоров'я України;
- Міністерство освіти і науки України;
- Національна академія наук України;
- Національна академія медичних наук України;
- Громадська організація «Всеукраїнська фармацевтична палата»;
- Харківська обласна державна адміністрація;
- Харківська обласна рада;
- Харківська міська рада;
- Громадська організація «Харківська обласна асоціація фармацевтичних працівників»;
- Державна служба України з лікарських засобів;
- Національний фармацевтичний університет

Шановні колеги!

Організаційний комітет запрошує Вас взяти участь у роботі VIII Національного з'їзду фармацевтів України, який відбудеться **13-16 вересня 2016 року** у м. Харкові на базі Національного фармацевтичного університету (посвідчення УкрІНТЕІ № 113 від 21.04.2015 р.).

У рамках проведення з'їзду відбудеться **науково-практична конференція «Фармація XXI століття: тенденції та перспективи».**

Мета з'їзду: підведення підсумків, обговорення та затвердження концепції розвитку фармацевтичного сектора галузі охорони здоров'я України на 2016-2021 рр.

Робочі мови з'їзду: українська, англійська, російська.

Делегати з'їзду обираються на регіональних конференціях згідно з Положенням і квотами, затвердженими Міністерством охорони здоров'я України та Фармацевтичною асоціацією України. Конференції щодо вибору делегатів проводяться регіональними асоціаціями фармацевтичних працівників **до 1 червня 2016 року**.

ОРІЄНТОВНА ПРОГРАМА З'ЇЗДУ

13 вересня 2016 року – реєстрація делегатів та учасників з'їзду, спонсорів, партнерів.

14 вересня 2016 року – урочисте відкриття VIII Національного з'їзду фармацевтів України, пленарні засідання, обговорення концепції розвитку фармацевтичного сектора галузі охорони здоров'я України на 2016-2021 рр.

15-16 вересня 2016 року – науково-практична конференція «Фармація XXI століття: тенденції та перспективи»: наукові симпозіуми, лекції майстер-класу, круглі столи, воркшопи, дискусії.

Організаційний внесок для одного делегата/учасника складає 995 грн (у тому числі ПДВ – 165 грн 83 коп.).

Організаційний внесок не передбачає оплати за проживання, але організаційний комітет зобов'язується розселити учасників з'їзду, якщо у реєстраційній формі Вами буде зроблена заявка. Інформація щодо проживання у готелях розміщена на сторінці з'їзду на сайті НФаУ.

Особи, які не є делегатами з'їзду, можуть взяти участь у його роботі (без права голосування) за умови сплати організаційного внеску. Їм гарантується участь у всіх заходах і отримання матеріалів нарівні з делегатами з'їзду.

Організаційний внесок гарантує:
- участь у пленарних засіданнях і науково-практичній конференції;
- одержання інформаційних і робочих матеріалів з'їзду;
- одержання делегатського кейсу;
- одержання ексклюзивних видань, підготовлених до VIII Національного з'їзду фармацевтів України;
- присутність на концертній програмі;

- участь у фуршетах під час роботи з'їзду;
- одержання сертифікату учасника з'їзду;
- участь в екскурсійній програмі;
- транспортні послуги.

Для участі тільки у **науково-практичній конференції «Фармація XXI століття: тенденції та перспективи» організаційний внесок для одного делегата складає** 400 грн (у тому числі ПДВ – 66 грн 67 коп.), що гарантує одержання інформаційних матеріалів VIII Національного з'їзду фармацевтів України, участь у роботі секційних засідань, наукових симпозіумів, круглих столів, лекціях майстер-класу, воркшопах, а також публікацію тез доповідей, одержання сертифікату учасника науково-практичної конференції.

Симпозіуми науково-практичної конференції

- Конструювання, синтез і модифікація біологічно активних сполук та створення на їх основі лікарських субстанцій.
- Сучасні підходи до створення нових лікарських та косметичних засобів, дієтичних добавок природного походження.
- Сучасний фармацевтичний аналіз та стандартизація ліків.
- Актуальні проблеми сучасної технології ліків, екстемпоральної рецептури, пакування та маркування лікарських препаратів.
- Сучасні аспекти розробки та промислового виробництва фармацевтичних препаратів. Біотехнології та нанотехнології у фармації.
- Механізми патологічних процесів та їх фармакологічна корекція.
- Клінічна фармація: від експериментальної розробки лікарських засобів до стандартизації фармацевтичної допомоги.
- Соціальна фармація: стан, проблеми та перспективи.
- Фармацевтична освіта в Україні.
- Фармація молода.

Публікація матеріалів

Матеріали науково-практичної конференції будуть опубліковані у збірнику матеріалів VIII Національного з'їзду фармацевтів України. Текст повідомлення (одна повна або дві повні сторінки) друкується на аркуші формату А 4 (ширина полів: ліве, праве, верхнє – по 2 см, нижнє – 3 см); шрифт Times New Roman, розмір шрифту – 12, інтервал – 1,1. Прохання дотримуватися наведеної структури:

Зверху по центру без відступу першого рядка:

НАЗВА ПОВІДОМЛЕННЯ ВЕЛИКИМИ ЛІТЕРАМИ (жирним шрифтом);

прізвище та ініціали авторів; якщо автор або один із співавторів повідомлення планує виступити на конференції з доповіддю, його прізвище слід підкреслити;

назва організації /наукової установи.

Через рядок друкується основний текст повідомлення (абзацний відступ – 1,25 см; вирівнювання по ширині, автоматичне розставляння переносів).

Усі матеріали подаються у 2-х примірниках і супроводжуються направленням від організації, в якій виконано роботу, експертним висновком, що дозволяє відкриту публікацію, та копією квитанції про оплату публікації матеріалів (або участь у з'їзді чи конференції). Другий примірник підписується всіма авторами. До друкованого варіанту матеріалів додається електронна копія – файл, виконаний у редакторі MS Word з розширенням RTF. Кожне повідомлення оформляється у вигляді окремого файлу, названого за прізвищем першого автора (якщо автор подає більше однієї роботи, до прізвища додається її порядковий номер). Файли слід надсилати разом з паперовим варіантом або електронною поштою доданим файлом, обов'язково вказуючи у темі повідомлення «Тези».

Оплата за публікацію однієї сторінки матеріалів складає 100 грн (у тому числі ПДВ 16,67 грн).

Особи, які сплатили організаційний внесок за участь у з'їзді або науковій конференції, звільняються від оплати за публікацію матеріалів.

Матеріали надсилати **не пізніше 1 червня 2016 р.** на адресу: 61002, м. Харків, вул. Пушкінська, 53, науковий відділ НФаУ, контактний телефон/факс: (057) 706-30-71, E-mail: conference_nauka@nuph.edu.ua (обов'язково вказувати у темі повідомлення «Тези»).

До уваги учасників!

Банківські реквізити для оплати:
р/р 26003060383169
МФО 351533, код ЄДРПОУ 33481466
Одержувач:
Громадська організація «Харківська обласна асоціація фармацевтичних працівників».
Призначення платежу:

- організаційний внесок за участь у VIII Національному з'їзді фармацевтів України;
- організаційний внесок за участь у науково-практичній конференції;
- за публікацію тез доповідей.

При сплаті обов'язково вказувати «у тому числі ПДВ».

ІНФОРМАЦІЯ ДЛЯ СПОНСОРІВ

З питань надання благодійної допомоги звертатися до відповідального секретаря VIII Національного з'їзду фармацевтів України.

Преференції спонсорам: можливість розповсюдження рекламної продукції фірми разом з інформаційними та робочими матеріалами з'їзду; розміщення логотипу спонсора на банерах та в усіх виданнях VIII Національного з'їзду фармацевтів України; можливість організації сателітних симпозіумів та освітніх заходів; участь у всіх заходах і отримання матеріалів з'їзду.

ОРГКОМІТЕТ VIII НАЦІОНАЛЬНОГО З'ЇЗДУ ФАРМАЦЕВТІВ УКРАЇНИ:

61002, м. Харків, вул. Пушкінська, 53, Національний фармацевтичний університет, відповідальний секретар оргкомітету проф. Зайченко Ганна Володимирівна.
Тел.: +38 (057) 706-22-69
Тел./факс: +38 (057) 706-30-98
E-mail: pharm_congress@nuph.edu.ua

СИНТЕЗ ТА АНАЛІЗ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН

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

UDC 547.789:543.51:543.544.5.068.7

OPTIMIZATION OF THE DETECTION CONDITIONS FOR THE SERIES OF 1,2,4-TRIAZOLE-3-THIONES FOR FIA-ESI-MS AND HPLC-ESI-MS

B.O.Varynskyi, A.G.Kaplaushenko

Zaporizhzhya State Medical University

Key words: flow injection analysis (FIA); high performance liquid chromatography (HPLC); mass-spectrometry (MS); electrospray ionization (ESI); 1,2,4-triazole-3-thions; optimization; polynomial regression

Development of methods for determination of 1,2,4-triazole-3-thiones as intermediate substances in the synthesis of 1,2,4-triazole-3-thioacetate acids salts as potential drugs is an important task at the research and production stage. The study of absorption, distribution, metabolism and excretion requires development of the corresponding methods. The most universal and selective methods used in these cases is the HPLC-MS. The aim of this study was to optimize the conditions of mass spectrometric detection for FIA-MS (flow injection analysis with mass-spectrometric detection) and HPLC-MS (high performance liquid chromatography with mass-spectrometric detection) with the electrospray ionization of eight 1,2,4-triazole-3-thiones by three factors of full factorial design and polynomial regression equations. The work has been performed using the technique of direct sample introduction into the ion source (flow injection analysis - FIA) on an Agilent 1260 Infinity HPLC system with an Agilent 6120 single quadrupole mass spectrometer. The equations of polynomial regression dependence of the signal intensity of the mass detector on three important factors such as the gas drier temperature, the fragmentor voltage and the nebulizer gas pressure have been calculated for the substances studied. Based on the location of the maxima of the functions obtained the optimal values of these factors have been determined. The choice of optimal conditions of mass spectrometric detection allows to maximize the signal on the detector and thus to increase the sensitivity and selectivity of determinations.

Currently, there are many derivatives of 1,2,4-triazoles with a high biological activity. They are potential or already widely used drugs. Development of control methods of synthetic stages for compounds of this class at the research and production stage is an important task for modern pharmaceutical science. Determining the ability of substances for absorption, distribution, metabolism and excretion (ADME) also needs to create approaches to the analysis of a number of compounds of this range. The high performance liquid chromatography with mass spectrometric detection, particularly with the electrospray ionization (HPLC-ESI-MS) is undoubtedly one of the universal and selective methods providing elucidation of the structure, determination of the molecular weight, quantitative determination of substances. Using the method HPLC-MS is the subject of many works, in which the conditions of mass spectrometric detection are given [5, 6, 8, 9], however, these 1,2,4-triazole-3-thiones have been studied for the first time, and therefore, the current study has relevance, scientific novelty and practical significance. We have not found papers concerning the use of polynomial models for choosing conditions of the electrospray ionization for mass spectrometric detection.

The aim was to optimize the conditions of ionization with electrospray for mass spectrometric detection of HPLC-MS range of 5-R-4-R1-1,2,4-triazoles-3-thiones as intermediates in the synthesis of the corresponding 1,2,4-triazoles-3-thioacetate acids and their salts, some of them have been already registered and some are potential medicines. In particular, these conditions are the gas drier temperature, the fragmentor voltage and the nebulizer gas pressure.

Experimental Part

The device for LC MS is the Agilent 1260 Infinity HPLC System (degasser, binary pump, autosampler, an Agilent 6120 single quadrupole mass spectrometer with ionization in electrospray (ESI); OpenLAB CDS Software. Conditions of the LC-MS study are: 1) the isocratic mode using the buffer solution: A, H_2O (HCOOH 0.1%) and solution of organic modifiers: B, CH_3CN (HCOOH 0.1%) – 50:50; 2) the ion source – API-ES; 3) the mode of selective ion monitoring depending on the molecular

Table 1

Full Factorial Design for determining the detection conditions of the test substances using ESI-MS

No. of the experiment	The gas drier temperature, T, ^{0}C	The fragmentor voltage, U, V	The nebulizer gas pressure, P, psi
1	100	0	10
2	100	0	30
3	100	0	60
4	100	150	10
5	100	150	30
6	100	150	60
7	100	300	10
8	100	300	30
9	100	300	60
10	200	0	10
11	200	0	30
12	200	0	60
13	200	150	10
14	200	150	30
15	200	150	60
16	200	300	10
17	200	300	30
18	200	300	60
19	300	0	10
20	300	0	30
21	300	0	60
22	300	150	10
23	300	150	30
24	300	150	60
25	300	300	10
26	300	300	30
27	300	300	60

weight, SIM; 4) positive polarity; 5) the gas drier rate (nitrogen) – 10 L/min.

Materials and Methods

Acetonitrile of the HPLC grade and formic acid (100%) were supplied by Merck KGaA (Darmstadt, Germany). Ultrapure water (18 MΩ cm at 25°C) was prepared using a Direct Q 3UV Millipore purification system (Molsheim, France).

The following compounds 4-(2-methoxyphenyl)-5-(pyridin-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**1**); 5-(furan-2-yl)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**2**); 5-(pyridin-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**3**); 5-(morpholin-4-ylmethyl)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4**); 4-methyl-5-(morpholin-4-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**5**); 4-ethyl-5-(morpholin-4-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**6**); 5-(morpholin-4-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**7**); 5-(2-methoxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**8**) were used. They were synthesized at the departments of Physical and Colloidal Chemistry (the head of the Dept. – Doctor of Pharmacy, associate professor Kaplaushenko A.G.), Toxicological and Inorganic Chemistry (the head of the Dept. – Doctor of Pharmacy, professor Panasenko O.I.) at the Zaporizhzhya State Medical University; their composition was confirmed earlier [1-4].

Preparation of Solutions

The sample of the substance solution was prepared by dissolving 1.0 mg of compounds **2, 4-7** in 1.0 mL of 50% acetonitrile. Analytes **1, 3** and **8** were dissolved in dimethyl sulphoxide (DMSO). The study was conducted by flow injection analysis (direct introduction of the sample into the ionization chamber without chromatographic separation). The injection volume was 2 μl.

Statistical Analysis

The statistical analysis of the results was performed on a personal computer employing Statistica Package v. 8.0 (StatSoft, USA) based on the values of full factorial design and the corresponding peak areas. The polynomial regression equations were determined. The optimal values of factors were found from the equations using Solver (optimization tool for Excel, Frontline Systems, Inc., USA).

Results and Discussion

Taking into account the opinions of developers in the research sector of hardware and software (Agilent Technologies company) presented in the recommendations of the OpenLAB CDS software system some of the factors of ionization for the mass detector were selected without optimization. According to these data we selected the flow rate of the eluent – 400 μl/min, the flow rate of the gas drier – 10 L/min, the capillary voltage – 4000 V before planning the experiment.

The objects under research are new derivatives of 1,2,4-triazoles, they have a specific structure, elemental composition, chemical bonds strength. Thus, we decided to optimize the factors affecting the value of the mass detector signal for each compound individually.

To achieve aim of the work the full factorial design by 3 factors was used. The interval for each factor was selected according to the recommendations of the OpenLAB CDS software package. Since the gas drier temperature recommended by the software is 300°C, but it also depends on the flow rate, the boiling point of the eluent, the gas drier rate and thermal stability of the sample. The eluent contained water, therefore, the temperature of 100°C was chosen as minimal. The nebulizer gas pressure was proposed by the software from 20 to 60 psi, depending on the flow rate of the eluent. As the minimal value 10 psi was chosen to study a broader range and determine the best value. The fragmentor voltage may vary depending on the nature of chemical bonds, so we varied it from 0 to 300 V. The flow-injection analysis (the method of the sample direct introduction) was used. The full factorial design is shown in Tab. 1. The factors were considered to be the best if they corresponded to the maximal value of the signal peak area of the mass detector. For each new compound of 1,2,4-triazole-3-thione series 27 combinations of factors (3^3) were studied. All in all 216 experiments were conducted. Each combination was repeated three times. Therefore, 648 measurements were done.

The equations of polynomial regression for dependence of the mass detector signal intensity on three factors, such as the gas drier temperature, the fragmentor

Table 2

The regression equations and the optimal conditions for mass spectrometric detection of the substances

Substances	SIM, m/z	Regression equations	R, R^2	Fcalc	$F_{f1,f2,\alpha}$	Optimal conditions		
						T	U	P
1	285	$S = 4118250.98 - 12777.609 \cdot T + 53.8893833 \cdot T^2 + 359928.242 \cdot U - 1260.7575 \cdot U^2 + 104089.817 \cdot P$	0.975171 0.950958	64.6	3.03	300	143	60
2	244	$S = 1565874.72 - 18586.382 \cdot T + 51.7684279 \cdot T^2 + 62410.7234 \cdot U - 216.66037 \cdot U^2 + 47798.1473 \cdot P$	0.955808 0.913569	35.2	3.03	300	144	60
3	179	$S = -2634692.0 + 23192.6314 \cdot T + 62.112728 \cdot T^2 + 637928.295 \cdot U - 2205.3596 \cdot U^2 + 517198.506 \cdot P - 4891.4904 \cdot P^2$	0.964899 0.931030	44.9	3.03	187	145	53
4	277	$S = 3410900.31 - 42541.095 \cdot T + 111.018572 \cdot T^2 + 122595.835 \cdot U - 458.85424 \cdot U^2 + 285964.863 \cdot P - 2930.0861 \cdot P^2$	0.894853 0.800762	13.4	3.03	300	134	49
5	215	$S = 2929281.14 + 5097.55089 \cdot T + 17.001536 \cdot T^2 + 59439.8118 \cdot U - 252.38307 \cdot U^2 + 133239.578 \cdot P - 1550.2213 \cdot P^2$	0.872500 0.761255	10.6	3.03	150	118	43
6	229	$S = 2421565.82 - 6703.0294 \cdot T + 16.2619500 \cdot T^2 + 37001.4610 \cdot U - 159.09844 \cdot U^2 + 68908.2841 \cdot P - 551.18155 \cdot P^2$	0.871407 0.759350	10.5	3.03	100	116	60
7	201	$S = -279018.59 + 9838.26968 \cdot T + 20.735148 \cdot T^2 + 59972.0000 \cdot U - 216.85533 \cdot U^2 + 56634.5387 \cdot P - 555.88194 \cdot P^2$	0.975704 0.951998	66.1	3.03	237	138	51
8	208	$S = 5354847.93 - 55171.225 \cdot T + 143.746231 \cdot T^2 + 43231.8132 \cdot U - 139.09561 \cdot U^2 + 36117.971 \cdot P + 496.813383 \cdot P^2$	0.728245 0.530340	3.76	3.03	300	155	10

voltage and the nebulizer gas pressure, were calculated. The adequacy of the models obtained was checked using Fisher criteria. The calculated value of Fisher's statistics F_{calc} for all compounds is more than F_{crit} of tabular values for $f_1 = 3$, $f_2 = 23$, $\alpha = 0.05$, and equals 3.03 indicating the significance of the polynomial regression equation. The coefficients of determination (R^2) for seven compounds are in the range of 0.76-0.95.

When calculating with the help of "Solver" for Excel the optimal values of the factors in determining the maxima of the functions were obtained, the search was converged in probability to a global solution.

The effect of the gas drier temperature on the mass detector signal intensity is generally described as follows: higher temperatures lead to more effective creation of ions, but at the definite temperature the thermal decomposition of molecules and ions of a substance increases [7]. Therefore, it is likely that the maximum intensity must be observed at the appropriate temperature. We found confirmation of this in practice.

Analysing the optimal temperature of the gas drier (Tab. 2) we note the following. Most compounds, which optimal temperature is 300°C, have the methoxyphenyl or phenyl radical (compounds **1, 2, 4**) in position 4 of the 1,2,4-triazole cycle, the exception is compound **8**, which has no substituents in position 4 of the 1,2,4-triazole cycle. It can be explained by the fact that introduction of the phenyl radical leads to π-π conjugation of the 1,2,4-triazole cycle, and it stabilizes the molecule as a whole. This fact correlates with the data obtained in the analysis of compounds **1** and **3** differing only by the presence of the 2-methoxyphenyl substituent in position 4 of the 1,2,4-triazole cycle of compound 1 and its absence for compound **3**, the optimal temperatures are 300°C and 187°C, respectively (the optimal temperature for compound **3** is below than that for compound **1**).

As for the results of the experiment with the substances containing morpholinomethylene substituents in position 5 of the 1,2,4-triazoles nucleus (compounds **4, 5, 6, 7**) it should be noted that the high temperature of 300°C is optimal for the compound containing phenyl in position 4 (compound **4**). For thione without substituent in N4 the best temperature is 237°C. Replacement of the methyl substituent to the ethyl one (compounds **5, 6**) creates conditions for reduction of the optimal temperature (from 150°C to 100°C). It can be explained by the fact that the phenyl radical stabilizes the compound through π-π conjugation with the 1,2,4-triazole cycle.

The absence of the substituent is characterized by the zero effect (**7**). The electron-donor properties of alkyl radicals increase (from thione **5** to **6**), and it destabilizes compounds.

Researchers from the Agilent technologies company carefully studied the correlation of the fragmentor voltage and the mass detector signal intensity [10]. It can be assumed that with increase in the fragmentor voltage the mass detector signal intensity should decrease because ions break up into fragments more actively. But, as we see from the data obtained by the authors [10], the signal intensity first increases, passes through the maximum, and then it decreases. The voltage on the capillary and the fragmentor applied to the inlet and outlet of the capillary significantly affect transmission of ions into the detector. The voltage also leads to fragmentation of the sample ions. Generally, the higher fragmentor voltage helps transmission of ions through the area of a relatively high pressure between the outlet of the capillary and the inlet to the skimmer. High fragmentor voltage can cause fragmentation that provides the structural information. For compounds that are fragmented with difficulty the increase in voltage usually results in better transmission of ions.

As can be seen from Table 2, the optimal conditions of fragmentation are in the range from 116 to 155 V. This factor depends on the structure of the compound. Thus, for morpholinomethylene derivatives (**4, 5, 6, 7**) the lowest optimal fragmentor voltage is observed, namely from 116 to 138 V. It can be seen that when shortening the alkyl chain alkyl chain on the methylene group (compounds **6, 5, 7**) the increase in the optimal fragmentor voltage can be observed, i.e. electron donor substituents reduce the stability of molecules. The presence of aromatic substituents stabilizes the molecule as a whole due to π-π conjugation with the triazole cycle (compounds **1-3, 7, 8**).

Prospects for further research. The next step is to study the chromatographic retention of these substances, as well as 1,2,4-triazole-3-thioacetate acids and their salts, as desired products.

CONCLUSIONS

1. The polynomial regression equations demonstrating dependence of the signal intensity of the mass detector on three important factors such as the gas drier temperature, the fragmentor voltage and the nebulizer gas pressure have been calculated for eight 1,2,4-triazole-3-thiones.

2. Based on the regression equations the optimal conditions of mass spectrometric detection of eight compounds have been calculated by three factors.

3. It has been determined that introduction of phenyl and methoxyphenyl substituents in position 4 of the 1,2,4-triazole cycle leads to greater stability of the compounds to temperature (the recommended temperature of the gas drier is 300°C). Introduction of the electron-donor alkyl substituent in position 4 reduces thermal stability when increasing the chain length; therefore, for ethyl derivatives the lowest temperature (100°C) is recommended.

4. Reduction of the methylene group in the alkyl chain (compounds **6, 5, 7**) increases the optimal fragmentor voltage, i.e. electron-donor substituents reduce the stability of molecules. The presence of aromatic substituents (compounds **1-3, 7, 8**) stabilizes the molecule as a whole due to π-π conjugation of the triazole cycle.

5. Determination of the optimal conditions for mass spectrometric detection is necessary to maximize the signal intensity on the detector and thus increase the sensitivity and selectivity of the methods. Since these conditions are somehow specific, the partial separation of the analyte signal from the signal of impurities is possible.

REFERENCES

1. *Каплаушенко А.Г. Синтез, будова і біологічна активність похідних 4-моно- та 4,5-дизаміщених 1,2,4-триазол-3-тіону: Автореф. дис. ... докт. фарм. наук.: 15.00.02. / Запорізький держ. мед. ун-тет, 2012. – 43 с.*
2. *Каплаушенко А.Г., Панасенко О.І., Книш Є.Г. та ін. // Фармац. журн. – 2008. – №2. – С. 67-72.*
3. *Самелюк Ю.Г., Каплаушенко А.Г. // Актуальні питання фармац. та мед. науки та практики. – 2013. – №2. – С. 125-128.*
4. *Щербіна Р.О., Панасенко О.І., Книш Є.Г. та ін. // Актуальні питання фармац. та мед. науки та практики. – 2014. – №3. – С. 18-21.*
5. *Cremin P., Kasim-Karakas S., Waterhouse A.L. // J. Agric. Food Chem. – 2001. – Vol. 49. – P. 1747-1750.*
6. *Gratz S.R., Flurer Ch.L., Wolnik K.A. // J. Pharmac. Biomed. – 2004. – Vol. 36. – P. 525-533.*
7. *Haverford college. Department of Chemistry. Resources. Instruments. Analysis by Direct Injection on the Agilent LC/MS. http://www.haverford.edu/chem/Agilent%20Direct%20Inject.pdf*
8. *Huang L.-F. // J. Pharmac. Biomed. – 2003. – Vol. 33. – P. 1155-1162.*
9. *Lin L.-Z. // J. Agric. Food Chem. – 2000. – Vol. 48. – P. 354-365.*
10. *Takino M. Determination of the metabolites of nitrofuran antibacterial drugs in chicken tissue by LC-ESI-MS, Agilent Technologies, 2003 (application note).*

ОПТИМІЗАЦІЯ УМОВ ДЕТЕКТУВАННЯ РЯДУ 1,2,4-ТРИАЗОЛ-3-ТІОНІВ ДЛЯ ПІА-ЕСІ-МС ТА ВЕРХ-ЕСІ-МС

Б.О.Варинський, А.Г.Каплаушенко

***Ключові слова**: проточно-інжекційний аналіз (ПІА); високоефективна рідинна хроматографія (ВЕРХ); мас-спектрометрія (МС); іонізація електророзпиленням (ЕСІ); 1,2,4-триазол-3-тіони; оптимізація; поліноміальна регресія*

Розробка методів визначення 1,2,4-триазол-3-тіонів як напівпродуктів синтезу солей 1,2,4-триазол-3-тіоацетатних кислот потенційних лікарських препаратів на стадії досліджень і виробництва є важливою задачею. Визначення поглинання, розподілу, метаболізму та виведення потребує розробки аналітичних методів. Найбільш універсальними і селективними методами, використаними в цих випадках, є ВЕРХ-МС. Мета цього дослідження полягала в оптимізації мас-спектрометричної детекції для ПІА-МС (проточно-інжекційний аналіз з мас-спектрометричною детекцією) і ВЕРХ-МС (високоефективної рідинної хроматографії з мас-спектрометричною детекцією) з іонізацією електророзпиленням восьми 1,2,4-триазол-3-тіонів по трьох факторах повного факторного експерименту і поліноміальних рівняннях регресії. Роботи проводилися з використанням методу прямого введення проби в іонне джерело за допомогою ВЕРХ системи Agilent 1260 Infinity з одноквадрупольним мас-спектрометром Agilent 6120. Були розраховані рівняння поліноміальної регресії залежності інтенсивності сигналу мас-детектора від трьох важливих факторів: температури газу-осушувача, напруги на фрагментаторі, тиску газу-розпилювача. На підставі максимумів функцій були отримані оптимальні значення цих факторів. Вибір оптимальних умов мас-спектрометричної детекції дозволяє максимізувати сигнал детектора і таким чином підвищити чутливість і селективність визначення.

ОПТИМИЗАЦИЯ УСЛОВИЙ ДЕТЕКТИРОВАНИЯ РЯДА 1,2,4-ТРИАЗОЛ-3-ТИОНОВ ДЛЯ ПИА-ЭСИ-МС И ВЭЖХ-ЭСИ-МС

Б.А.Варинский, А.Г.Каплаушенко

***Ключевые слова:** проточно-инжекционный анализ (ПИА); высокоэффективная жидкостная хроматография (ВЭЖХ); масс-спектрометрия (МС); ионизация электрораспылением (ЭСИ); 1,2,4-триазол-3-тионы; оптимизация; полиномиальная регрессия*

Разработка методик определения 1,2,4-триазол-3-тионов как промежуточных веществ при синтезе солей 1,2,4-тризолтиоацетатных кислот потенциальных лекарственных веществ на исследовательском и производственном этапе является важной задачей. Исследование адсорбции, распределения, метаболизма и экскреции требует создания соответствующих методик. Наиболее универсальные и селективные методы, применяемые в этих случаях, это ВЭЖХ-МС.Целью этого исследования была оптимизация условий масс-спектрометрического детектирования для ПИА-МС (проточно-инжекционный анализ с масс-спектрометрической детекцией) и ВЭЖХ-МС (высокоэффективная хроматография с масс-спектрометрической детекцией) с ионизацией в электроспрее (ЭСИ) восьми 1,2,4-триазол-3-тионов по 3 факторам полного факторного эксперимента и полиномиальным уравнениям регрессии. Работа была проделана техникой прямого ввода образца в ионный источник на Agilent 1260 Infinity HPLC system с одноквадрупольным масс-спектрометром Agilent 6120.Были рассчитаны уравнения полиномиальной регрессионной зависимости сигнала масс-детектора от трех важных факторов: температуры газа осушителя, напряжения на фрагментаторе, давления газа небулайзера. Оптимальные значения этих факторов были рассчитаны на основе определения максимумов полученных функций. Выбор оптимальных условий масс-спектрометрической детекции позволяет максимизировать сигнал на детекторе и таким образом увеличить чувствительность и селективность определений.

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

UDC 543.637:547-304.3

THE REACTIVITY OF AROMATIC AND HETEROCYCLIC DERIVATIVES OF HYDRAZINE. VII. THE ACID-BASE PROPERTIES OF SUBSTITUTED 5,7-DICHLORO-9-HYDRAZINE ACRIDINE

A.O.Devyatkina, O.M.Svechnikova, S.V.Kolisnyk, N.P.Kobzar, O.F.Vinnik

National University of Pharmacy

Kharkiv National Pedagogical University named after G.S.Skovoroda

Key words: substituted 5,7-dichloro-9-hydrazine acridine; reactivity; Hammet correlation equation

The reactivity of substituted 5,7-dichloro-9-hydrazine acridine that exhibit various pharmacological activity by studying acid-base equilibria in the ethanol-water binary solvent (50 Mol % ethanol) at 25°C using the method of potentiometric titration has been investigated. The values pK of the corresponding conjugate acids obtained for 7 compounds indicate that these compounds are very weak bases. Analysis of the impact of the nature and position of substituents in the heterocycle on the strength of the corresponding conjugate acids has shown that the acceptor substituents weaken basicity of 5,7-dichloro-9-hydrazine acridine, and donor substituents cause the opposite effect. The quantitative assessment of the influence of substituents in the molecule by the Hammett equation within the principle of linearity of free energies with convincing statistical parameters has shown a low sensitivity of the reaction centre to structural changes in the molecule of 5,7-dichloro-9-hydrazine acridine. Using the correlation equation and the value pK_{BH^+} experimentally obtained for 1-CH_3 substituent the σ-constant of this substituent: σ(1-CH_3) = 0.056 has been determined. The Hammett correlation equation is used to predict the acid-base properties of substituted 5,7-dichloro-9-hydrazine acridines and the molecular design of more active pharmacophores.

Substituted 9-hydrazine acridines exhibit the antimicrobial, antihypoxic, analgesic activity [3, 5, 6, 9-11] and are precursors for the synthesis of their various derivatives with new pharmacological properties. Therefore, the study of their reactivity is of undoubted scientific and practical interest since it allows optimizing the ways of their synthesis and predicting the biological activity.

Materials and Methods

The synthesis of substituted 5,7-dichloro-9-hydrazine acridine (**1-7**) was carried out according to the method [11], their physicochemical parameters are shown in Table.

The ionization constants of the compounds under research were determined by the method of potentiometric titration [1, 7, 17] in the ethanol-water binary solvent (50 Mol % ethanol) at 25°C. These constants are given in Table.

The study of acid-base equilibria was carried out by the method [8]. The titrant was 0.01 M aqueous solution of HCl. The concentration of the titrated solutions was 0.005 Mol·dm^{-3} at the semineutralization point. Potentiometric titration was performed on an EB-74 ionomer with glass (ESP-43-074) and silver-silver chloride electrodes at 25°C. The experiment was performed in triplicates and processed in accordance with the requirements of the SPhU [2]. The correlation analysis was conducted by the method of mathematical statistics (the confidence probability – 0.95) [2, 8].

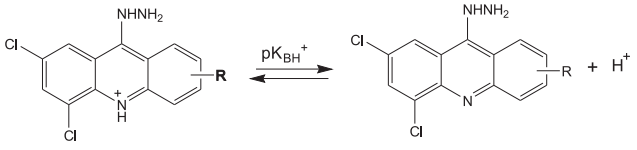
To prepare the mixed solvent the CO_2-free bidistillate and ethanol were used.

Results and Discussion

The reactivity of substituted 5,7-dichloro-9-hydrazine acridine of this isostructural series was studied in reversible conditions. Ionization of NH-acids conjugated with substituted 5,7-dichloro-9-hydrazine acridine was studied according to the equation (Scheme).

The preliminary experiments have proven that on the pH – f (V_{HCl}) plot there is only one point of intersection that coincides with the literature data [4, 12-15] about protonation of 9-hydrazine acridine only on the heterocyclic nitrogen.

The data in Table indicate that the compounds studied are very weak bases in contrast to substituted 9-aminoacridines [12]. This suggests the lack of resonance stabilization of the cation of 9-hydrazine acridine due to the isolating effect of the imino group. The introduction of acceptor substituents increases the positive charge at the reaction centre, therefore, the strength of conjugate acids with the acceptor substituent (2-NO_2, 4-NO_2) regularly increases compared to the unsubstituted acid (the strength of the corresponding base reduces). Donor substituents cause the opposite effect.

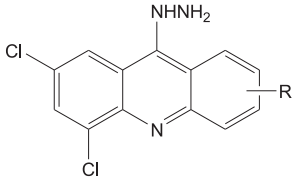


Scheme

where: R = -H, 1-CH_3, 2-CH_3, 3-CH_3, 4-CH_3, 2-NO_2, 4-NO_2.

Table

The properties of substituted 5,7-dichloro-9-hydrazine acridine



Compound	R	Yield, %	M. p., °C	Found,%			Mol. formula	Calc.,%			pK_{BH^+}
				C	N	H		C	N	H	
1	H	78	175-178	56.37	15.18	2.92	$C_{13}H_8Cl_2N_3$	56.34	15.16	2.91	4.11±0.02
2	1-CH_3	75	190-193	57.53	14.40	3.77	$C_{14}H_{11}Cl_2N_3$	57.55	14.38	3.79	4.15±0.03
3	2-CH_3	77	204-205	57.58	14.41	3.80	$C_{14}H_{11}Cl_2N_3$	57.55	14.38	3.79	4.15±0.02
4	3-CH_3	74	214-217	57.59	14.36	3.78	$C_{14}H_{11}Cl_2N_3$	57.55	14.38	3.79	4.14±0.01
5	4-CH_3	75	185-188	57.51	14.33	3.77	$C_{14}H_{11}Cl_2N_3$	57.55	14.38	3.79	4.13±0.01
6	2-NO_2	72	238-241	48.29	17.38	2.50	$C_{13}H_8Cl_2N_4O_2$	48.32	17.34	2.49	3.74±0.02
7	4-NO_2	73	220-223	48.30	17.37	2.48	$C_{13}H_8Cl_2N_4O_2$	48.32	17.34	2.49	3.79±0.02

The quantitative assessment of the influence of substituents in the molecule of 5,7-dichloro-9-hydrazine acridine was carried out by the Hammett equation within the principle of linearity of free energies.

Correlation occurred with all data except pK_{BH^+} (1-CH_3). For this substituent the σ-constant is absent. The correlation equation obtained has reliable statistical parameters, indicating the reliability of prediction:

$$pK_{BH^+} = (4.11\pm0.04) - (0.72\pm0.01) \cdot \sigma$$
$$n=6 \qquad r=0.998 \qquad s=0.054.$$

The reaction constant ρ in the equation is small by the absolute value (ρ = 0.72), and it indicates the low sensitivity of the reaction centre to structural changes in the molecule of 5,7-dichloro-9-hydrazine acridine.

The plot of pK_{BH^+} – f(σ) is linear (Fig.)

Using the correlation equation and the value pK_{BH^+} experimentally obtained for 1-CH_3 substituent the σ-constant of this substituent (Table) – σ(1-CH_3) = 0.056 was determined.

It is interesting to note that the reaction constant ρ for substituted 5,7-dichloro-9-hydrazine acridine within the experimental error coincides with ρ for substituted 9-aminoacridine [16], it indicates a single mechanism of electronic effects of substituents on the reaction centre.

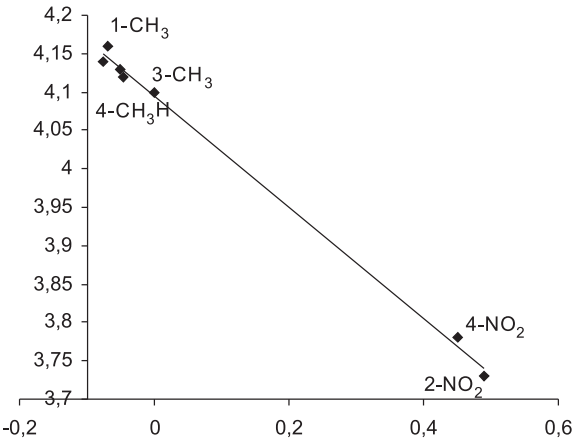


Fig. The plot of pK_{BH^+} – f(σ) for substituted 5,7-dichloro-9-hydrazine acridine.

CONCLUSIONS

1. The reactivity of substituted 5,7-dichloro-9-hydrazine acridine has been studied in reversible conditions by investigating acid-base equilibria of conjugate acids in the mixed ethanol-water solvent (50 Mol % ethanol) at 25°C.

2. The impact of the nature and position of substituents in the heterocycle on the strength of the corresponding conjugate acids has been analyzed.

3. It has been proven that the acceptor substituents weaken basicity of 5,7-dichloro-9-hydrazine acridine, and donor substituents cause the opposite effect.

4. The Hammett correlation equation with convincing statistical characteristics has been determined, it is used to predict the acid-base properties of substituted 5,7-dichloro-9-hydrazine acridines.

REFERENCES

1. *Альберт А., Сержент Е. Константы ионизации кислот и оснований. – М.: Химия, 1964. – 178 с.*
2. *Державна фармакопея України / Державне підприємство «Науково-експертний фармакопейний центр». – 1-е вид. – Х.: РІРЕГ, 2001. – 556 с.*
3. *Ісаєв С.Г. // Ліки. – 2001. – №3/4. – С. 72-74.*
4. *Ісаєв С.Г., Свєчнікова О.М., Павлій О.І. // Вісник фармації. – 2003. – №4 (36). – С. 27-29.*
5. *Ісаєв С.Г. // Фармац. журн. – 1999. – №3. – С. 52-54.*

6. *Ісаєв С.Г., Яременко В.Д., Русакова Н.П. // Фармац. журн. – 2000. – №1. – С. 72-75.*
7. *Лабораторное руководство по хроматографии и смежным методам / Пер. с англ. – М.: Мир, 1982. – Т. 1. – 396 с.*
8. *Львовский Е.Н. Статистические методы построения эмпирических формул. – М.: Высш. шк., 1988. – 125 с.*
9. *Мартиновський О.О., Панасенко Т.В., Панасенко О.І. // Вісник фармації. – 1999. – №2 (20). – С. 44-46.*
10. *Мохамед Мохамед Мохмуд Барака. Синтез, физико-химические свойства и биологическая активность тио-, гидразинопроизводных 6-хлор-7-нитроакридина: Автореф. дис. … канд. фармац. наук. – Х., 1992. – 19 с.*
11. *Панасенко А.Н., Омельянчик Л.А., Панасенко Т.В. та ін. // Актуальні питання фармацевтичної та медичної науки та практики. – Запоріжжя, 1988. – Т. 2, вип. 2. – С. 60-63.*
12. *Свечникова Е.Н. // ЖОХ. – 1997. – Т. 67, вып. 1. – С. 138-140.*
13. *Свечникова Е.Н. // ЖОХ. – 1998. – Т. 67, вып. 6. – С. 1007-1008.*
14. *Свечникова Е.Н. // ЖОХ. – 1999. – Т. 68, вып. 5. – С. 865-866.*
15. *Свечникова Е.Н. // ЖОХ. – 2001. – Т. 71, вып. 5. – С. 848-849.*
16. *Свєчнікова О.М. Реакційна здатність, зв'язок структура-біологічна активність та використання похідних N-фенілантранілової кислоти та акридину: Автореф. дис. … докт. хім. наук. – Х., 1999. – 35 с.*
17. *Таблицы констант скорости и равновесия гетеролитических органических реакций. – М.: ВИНИТИ, 1975. – Т. 1. – 396 с.*

РЕАКЦІЙНА ЗДАТНІСТЬ АРОМАТИЧНИХ ТА ГЕТЕРОЦИКЛІЧНИХ ПОХІДНИХ ГІДРАЗИНУ. VII. КИСЛОТНО-ОСНОВНІ ВЛАСТИВОСТІ ЗАМІЩЕНИХ 5,7-ДИХЛОРО-9-ГІДРАЗИНОАКРИДИНУ

А.О.Девяткіна, О.М.Свєчнікова, С.В.Колісник, Н.П.Кобзар, О.Ф.Вінник

***Ключові слова**: заміщені 5,7-дихлоро-9-гідразиноакридину; реакційна здатність; кореляційне рівняння Гаммета*

Досліджена реакційна здатність заміщених 5,7-дихлоро-9-гідразиноакридину, що проявляють різноманітну фармакологічну активність, шляхом вивчення кислотно-основних рівноваг у бінарному розчиннику етанол-вода (50 мольних % етанолу) при 25°С методом потенціометричного титрування. Одержані значення pK відповідних спряжених кислот для 7 сполук свідчать, що ці сполуки є вельми слабкими основами. Аналіз впливу природи і положення замісників у гетероциклі на силу відповідних спряжених кислот показав, що акцепторні замісники послаблюють основність 5,7-дихлоро-9-гідразиноакридину, а донорні чинять протилежний вплив. Кількісна оцінка впливу замісників у молекулі за рівнянням Гаммета у межах принципу лінійності вільних енергій з переконливими статистичними параметрами показала низьку чутливість реакційного центру до структурних змін у молекулі 5,7-дихлоро-9-гідразиноакридину. З використанням одержаного кореляційного рівняння і експериментально одержаного значення pK_{BH^+} для 1-CH_3 заміщеного визначена σ-константа цього замісника: σ(1-CH_3) = 0,056. Кореляційне рівняння Гаммета використовується для прогнозування кислотно-основних властивостей заміщених 5,7-дихлоро-9-гідразиноакридинів та молекулярного дизайну більш активних фармакофорів.

РЕАКЦИОННАЯ СПОСОБНОСТЬ АРОМАТИЧЕСКИХ И ГЕТЕРОЦИКЛИЧЕСКИХ ПРОИЗВОДНЫХ ГИДРАЗИНА. VII. КИСЛОТНО-ОСНОВНЫЕ СВОЙСТВА ЗАМЕЩЕННЫХ 5,7-ДИХЛОР-9-ГИДРАЗИНОАКРИДИНА

А.А.Девяткина, Е.Н.Свечникова, С.В.Колесник, Н.П.Кобзарь, А.Ф.Винник

***Ключевые слова**: замещенные 5,7-дихлор-9-гидразиноакридина; реакционная способность; корреляционное уравнение Гаммета*

Исследована реакционная способность замещенных 5,7-дихлор-9-гидразиноакридина, проявляющих разнообразную фармакологическую активность, путем изучения кислотно-основных равновесий в бинарном растворителе этанол-вода (50 мольных % этанола) при 25°С методом потенциометрического титрования. Полученные значения pK соответствующих сопряженных кислот для 7 соединений свидетельствуют, что эти соединения являются слабыми основаниями. Анализ влияния природы и положения заместителей в гетероцикле на силу соответствующих сопряженных кислот показал, что акцепторные заместители ослабляют основность 5,7-дихлор-9-гидразиноакридина, а донорные оказывают противоположное влияние. Количественная оценка влияния заместителей в молекуле по уравнению Гаммета в рамках принципа линейности свободных энергий с убедительными статистическими параметрами показала низкую чувствительность реакционного центра к структурным изменениям в молекуле 5,7-дихлор-9-гидразиноакридина. С использованием полученного корреляционного уравнения и экспериментально определенного значения pK_{BH^+} для 1-CH_3 замещенного определена σ-константа этого заместителя: σ(1-CH_3) = 0,056. Корреляционное уравнение Гаммета используется для прогнозирования кислотно-основных свойств замещенных 5,7-дихлор-9-гидразиноакридинов и молекулярного дизайна более активных фармакофоров.

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

UDC 54.062:616.31:547.82:543.24

DEVELOPMENT OF THE QUANTITATIVE DETERMINATION METHOD FOR A NEW CARIES-PREVENTIVE COMPOUND

V.Yu.Anisimov, V.O.Gelmboldt, N.Yu.Bevz, V.A.Georgiyants

Odessa National Medical University

National University of Pharmacy

Key words: pharmaceutical analysis; quantitative determination; validation of the analytical method; cetylpyridinium hexafluorosilicate; caries-preventive agent

Fluorides are the most important treatment and preventive additive in the composition of any form; they prevent development of caries by increasing the resistance of enamel, as well as production of acids by bacteria of dental plaque. At the Odessa National Medical University the work on searching for fluorine-containing compounds in a series of quaternary bases and their subsequent use in dentistry is conducted. The pharmacological studies have shown that "onium" hexafluorosilicates have a higher caries-preventive effectiveness compared to sodium fluoride. Cetylpyridinium hexafluorosilicate has been found to be the most active in the dose of 15 mg/kg when used in the form of oral applications of the gel; its mechanism of action is in activation of alkaline phosphatase and lysozyme of the pulp of the teeth. Development of reliable methods for identification and quantitative determination is a prerequisite for further use of this compound in medical practice. The aim of this work was to develop the method for quantitative determination of cetylpyridinium hexafluorosilicate. For further use of the method proposed for analysis of the compound under research its validation characteristics have been studied. According to the results of the research conducted it has been found that the method for quantitative determination of cetylpyridinium hexafluorosilicate in the substance corresponds to the following parameters: accuracy, precision, linearity (Δ_z = 0.50≤max Δ_z = 0.53, δ = 0.17≤max δ = 0.32, a = 0.80≤max a = 1.60, r = 1.0000≥min r = 0.9993).

Over the past decade there is a significant increase of affected teeth by caries in the population [8]. Caries is a disease, in which under the effect of bacteria the process of demineralization of teeth occurs. The risk of caries is associated with a number of causes, among them there is deficiency of fluorine in food and drinking water, which leads to brittleness and thinning of the enamel; the excess of carbohydrate food and sugar; dental plaque formed from decomposition of food debris; and it is also a stimulus for bacterial growth. In turn, excessive amounts of fluoride lead to binding of calcium salts in the inert calcium fluoride and the hepatotoxic action. Hexafluorosilicates (SiF_6) are one of the fixed forms of fluorine; moreover, they are almost completely free of drawbacks of fluorides [7]. In order to find substances with the caries-protective and antibacterial properties the work on searching for new biologically active substances among hexafluorosilicate derivatives is conducted at the Odessa National Medical University [5, 6].

One of the most active compounds in this series is the salt of the quaternary base – cetylpyridinium hexafluorosilicate; development of methods of the quality control is the necessary condition for its further application. The basic physical and chemical properties of cetylpyridinium hexafluorosilicate have been studied, and the methods for its identification have been proposed [4].

Continuing the research on the standardization of the compound it was necessary to develop a method for its quantitative determination.

Materials and Methods

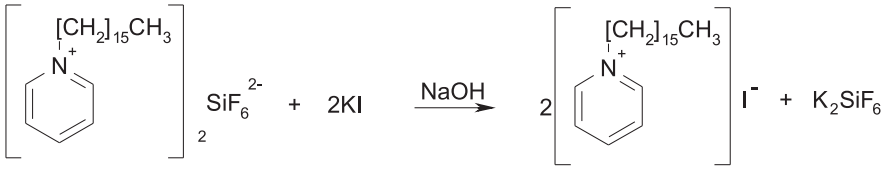
The experiments were carried out using the chromatographic grade sample of the compound (the content of impurities – 0.5%). During the work the measuring glassware of class A, reagents meeting the requirements of the State Pharmacopoeia of Ukraine (SPhU) and "AXIS" analytical balances were used.

The quantitative determination method. Dissolve 2.000 g in distilled water and dilute to 100.0 ml with the same solvent. Transfer 25.0 ml of the solution to a separating funnel, add 25 ml of chloroform, 10 ml of 0.1 M sodium hydroxide and 10.0 ml of a freshly prepared 50 g/l solution of potassium iodide. Shake well, allow to separate the chloroform layer and discard chloroform extracts. To the aqueous layer add 40 ml of hydrochloric acid, cool and titrate with 0.05 M potassium iodate to a deep-brown colour that does not disappear. Add 2 ml of chloroform and continue to titrate, shaking vigorously, until the chloroform layer no longer changes its colour. Simultaneously carry out the blank titration of a mixture of 10.0 ml of the freshly prepared 50 g/l solution of potassium iodide, 20 ml of water and 40 ml of hydrochloric acid.

One ml of 0.05 M solution of potassium iodate corresponds to 37.56 mg of $(C_{21}H_{38}N)_2SiF_6$, which must be from 99.0% to 101.0%.

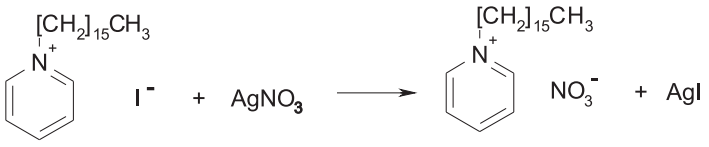
Results and Discussion

Cetylpyridinium hexafluorosilicate is a quaternary ammonium salt, the residue of the pyridine cycle is in the basis of its structure. Cetylpyridinium hydrochloride is



$$2KI + KIO_3 + 6HCl \rightarrow 3ICl + 3KCl + 3H_2O$$

Scheme 1



Scheme 2

Table 1

Accuracy and convergence of the results of quantitative determination

No. of the model solution	The substance amount in the sample	Introduced in % to the concentration of the reference solution (Xiact%)	The volume of 0.05 M solution of KIO_3, V, ml (K = 1.0000)	Found in % to the concentration of the reference solution (Yi%)	Found in % to the introduced Zi = 100 (Yi/Xi)
1	0.1610	80.50	27.00	80.94	100.54
2	0.1708	85.40	26.60	85.27	99.85
3	0.1815	90.75	26.05	91.86	101.22
4	0.1912	95.60	25.65	95.21	99.60
5	0.2018	100.90	25.10	100.66	99.76
6	0.2107	105.35	24.60	105.50	100.14
7	0.2207	110.35	24.05	110.27	99.93
8	0.2309	115.45	23.45	115.36	99.92
9	0.2402	120.10	22.85	120.47	100.31
Mean, Z%					100.14
Relative standard deviation, Sz%					0.50
Relative confidence interval Δ_{As}% = t (95%,7)×Sz					0.53
Critical value for convergence of results Δ_{As}%					1.00
Systematic error δ					0.17
Criterion of the systematic error insignificance 1) δ≤Δ_{As}/(g)^0.5 = 0.33, 2) if not satisfied 1), then δ≤0.33					0.32
The overall conclusion of the method					correct

analogue by the structure of the compound studied. For its quantitative assessment the European and British Pharmacopoeias recommend to use the method of titration with potassium iodate after the appropriate sample preparation. First, the solution of potassium iodide in the alkaline medium was added, and the resulting compound was extracted with chloroform. The excess of potassium iodide in the aqueous layer was determined after acidifying the reaction mixture by titration with 0.05 M solution of potassium iodate [2, 3].

We assumed that when adding potassium iodide cetylpyridinium hexafluorosilicate in the alkaline medium formed cetylpyridinium iodide having the properties of the ion associate and being well extracted with chloroform.

We consider that the reaction proceeds by the following mechanism (Scheme 1).

We confirmed that the reaction occurred by the exactly this mechanism in the following way. The chloroform layer was carefully evaporated on a water bath to dryness, suspended with water, acidified by acetic acid diluted to a yellow-green colour with the bromphenol blue indicator. While thoroughly stirring the reaction mixture was titrated slowly with 0.1 M solution of silver nitrate to an emerald-green colour. In the process of titration the precipitate was dissolved, and colloidal precipitate of silver iodide was gradually formed (Scheme 2).

Some validation characteristics of the method proposed for titration of the cetylpyridinium hexafluorosilicate substance with the indicator fixation of the titration end point were studied according to the requirements of the SPhU [1]. To validate the titration method the experimental batch of the substance was used. The loss on drying was 2.0%. In calculations the content of the active substance was taken equal to 100%.

To reduce uncertainty the titre of 0.05 *M* potassium iodide solution was determined by the method of the SPhU [1].

Table 2

The results of the linear dependence determination

Parameters	Values	Criteria (for tolerances 99-101%, g = 9)	Conclusion (satisfied or not)
b	0.9931	–	–
Sb	0.0091	–	–
a	0.8016	max a = 1.60	satisfied
Sa	0.9255	–	–
So	0.3509	max S_o = 0.53	satisfied
r	1.0000	min r = 0.99926	satisfied
RSD range	13.5805	–	–
Ro	0.9997	–	–
Sb^2	0.0001	–	–
Sa^2	0.8566	–	–
$\delta_{RL,80} = 100 \cdot \left\| \frac{a}{80} + (b-1) \right\| \le \frac{2}{3} \cdot \max \Delta_{As} \le 0.6600$	0.3308		
$\delta_{RL,120} = 100 \cdot \left\| \frac{a}{120} + (b-1) \right\| \le \frac{2}{3} \cdot \max \Delta_{As} \le 0.6600$	0.0031		
DL	3.0748		
LOQ	9.3193		

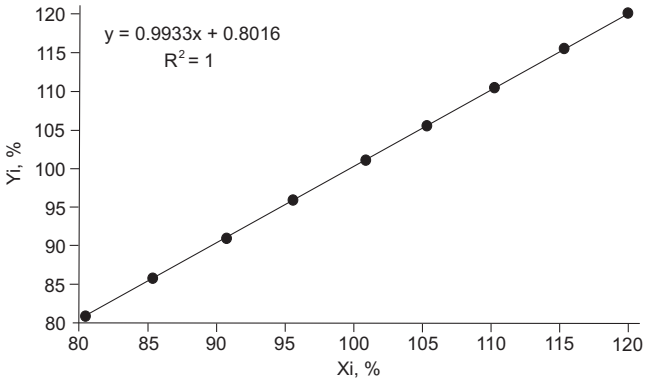


Fig. The linear dependence of 0.05 M solution of potassium iodate on the concentration of the compound studied.

The mean value of 5 parallel titrations was obtained. The value of the correction factor to the nominal concentration of the titrated solution K_T was 1.0000 with the relative standard deviation *RSD* = 0.10% and the confidence interval Δ*(titr)* = 0.10%. Thus, the results of the titre determination (≤0.2%) comply with the requirements of the SPhU for convergence [1].

The titration was carried out after adding a certain amount of potassium iodide solution, hydrochloric acid and chloroform; therefore, to reduce the error of titration it was appropriate to conduct the blank titration simultaneously.

To determine linearity the samples were taken for different points (*i*) of the straight line, they were 80, 85, 90, 95, 100, 105, 110, 115 and 120% from the nominal weight of 200 mg. To study the reproducibility of the results for different experiments for studying linearity 5 samples for each point (*i*) were taken (Tab. 1).

The results obtained were processed by the least squares method. The values X_i, Y_i and Z_i are given in Tab. 1.

The results of the linear dependence processing by the least square method are given in Tab. 2 and Fig. As can be seen from Tab. 2, the requirement of simultaneous statistical insignificance of the values │a│ and │1-b│ is performed for the set of 9 points; it meets the requirements of the practical acceptance of the linear dependence.

It should be noted that the systematic error value both by 80% of the nominal content ($\delta_{RL,80}$), and by 120% ($\delta_{RL,120}$) does not exceed the maximal value (Tab. 2). The determination limit (DL) and limit of quantification (LOQ) do not exceed 32%, i.e. they do not significantly affect the quantitative determination (Tab. 2).

From these calculations it is apparent that the maximum permitted value of the complete predicted uncertainty of the analytical procedure is more than the total calculated uncertainty of the method developed for quantitative determination of the active ingredient in the substance. Therefore, the method of redox titration can be used for quantitative determination of cetylpyridinium hexafluorosilicate with the tolerance of the active substance content of ±1.0%.

CONCLUSIONS

The method for quantitative determination of cetylpyridinium hexafluorosilicate in the substance has been developed using the redox method. When determining the basic validation characteristics of the method specified it has been found that the requirements for linearity, precision, accuracy are performed, and this method can be recommended for use.

REFERENCES

1. *Державна фармакопея України: в 3-х т. / Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів». – 2-е вид. – Х.: Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів», 2015. – Т. 1. – 1128 с.*
2. *British Pharmacopoeia [Електронний ресурс] / The British Pharmacopoeia Secretariat. – London, 2009. – Vol. II. – Режим доступу: https://www.vek-com.ru/78022.html.*
3. *European Pharmacopoeia 6th ed. – Strasbourg: European Directorate for the Quality of Medicines, 2008. – Vol. 2. – 3308 p.*
4. *Gelmboldt V.O., Anisimov V.Yu., Bevz N.Yu., Georgiyants V.A. // Der Pharma Chemica. – 2016. – Vol. 8, №1. – P. 169-173.*
5. *Gelmboldt V.O., Ganin Ed.V., Fonari M.S. et al. // J. Fluorine Chem. – 2009. – Vol. 130. – P. 428-433.*
6. *Gelmboldt V.O., Prodan O.V., Anisimov V.Yu. // Am. J. of Pharm. Tech. Res. – 2014. – Vol. 4, № 6. – P. 513-521.*
7. *Lepsky V.V., Anisimov V.Yu., Lepsky V.V. // J. of Education, Health and sport. – 2015. – Vol. 5, №11. – P. 289-299.*
8. *Levitsky A.P., Gorokhivskiy V.N., Selivanskaya I.A. // Aktual'nye problemy transportnoy meditsiny. – 2014. – Vol. 3, №37. – P. 136-139.*

РОЗРОБКА МЕТОДИКИ КІЛЬКІСНОГО ВИЗНАЧЕННЯ НОВОЇ КАРІЄС-ПРОФІЛАКТИЧНОЇ СПОЛУКИ

В.Ю.Анісімов, В.О.Гельмбольдт, Н.Ю.Бевз, В.А.Георгіянц

***Ключові слова**: фармацевтичний аналіз; кількісне визначення; валідація аналітичної методики; цетилпіридинію гексафлуоросилікат; карієс-профілактичний засіб*

Флуориди є найважливішою лікувально-профілактичної добавкою в складі будь-якої форми і запобігають розвитку карієсу, підвищуючи стійкість емалі, та перешкоджають виробленню кислот бактеріями зубного нальоту. У Одеському національному медичному університету проводиться робота з пошуку флуоровмісних сполук у ряду четвертинних основ з подальшим їх застосуванням у стоматології. Фармакологічні дослідження довели, що «онієві» гексафлуоросилікати мають більш високу карієс-профілактичну ефективність в порівнянні з натрію флуоридом. Найбільш активним виявився цетилпіридинію гексафлуоросилікат в дозі 15 мг/кг при використанні у вигляді оральних аплікацій гелю, механізм дії якого полягає в активації лужної фосфатази і лізоциму пульпи зубів. Для подальшого застосування сполуки в медичній практиці, необхідною умовою є розробка надійних методик його ідентифікації та кількісного визначення. Метою роботи стала розробка методики кількісного визначення цетилпіридинію гексафлуоросилікату. Для подальшого застосування запропонованої методики для аналізу досліджуваної сполуки, вивчали валідаційні характеристики. За результатами проведених досліджень встановлено, що методика кількісного визначення цетилпіридинію гексафлуоросилікату в субстанції відповідає за параметрами: правильність, прецизійність, лінійність (Δ_z = 0.50≤max Δ_z = 0.53, δ = 0.17≤max δ = 0.32, a = 0.80≤max a = 1.60, r = 1.0000≥min r = 0.9993).

РАЗРАБОТКА МЕТОДИКИ КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ НОВОГО КАРИЕС-ПРОФИЛАКТИЧЕСКОГО СОЕДИНЕНИЯ

В.Ю.Анисимов, В.О.Гельмбольдт, Н.Ю.Бевз, В.А.Георгиянц

***Ключевые слова**: фармацевтический анализ; количественное определение; валидация аналитической методики; цетилпиридиния гексафторосиликат; кариес-профилактическое средство*

Фториды являются важнейшей лечебно-профилактической добавкой в составе любой формы и предотвращают развитие кариеса, повышая стойкость эмали, и препятствуют выработке кислот бактериями зубного налета. В Одесском национальном медицинском университете проводится работа по поиску фторсодержащих соединений в ряду четвертичных оснований с последующим их применением в стоматологии. Фармакологические исследования показали, что «ониевые» гексафторосиликаты имеют более высокую кариес-профилактическую эффективность по сравнению с натрия фторидом. Наиболее активным оказался цетилпиридиния гексафторосиликат в дозе 15 мг/кг при использовании в виде оральных аппликаций геля, механизм действия которого заключается в активации щелочной фосфатазы и лизоцима пульпы зубов. Для дальнейшего применения соединения в медицинской практике, необходимым условием является разработка надежных методик его идентификации и количественного определения. Целью работы стала разработка методики количественного определения цетилпиридиния гексафторосиликата. Для дальнейшего использования предложенной методики для анализа исследуемого соединения, изучали валидационные характеристики. По результатам проведенных исследований установлено, что методика количественного определения цетилпиридиния гексафторосиликата в субстанции соответствует по параметрам: правильность, прецизионность, линейность (Δ_z = 0.50≤max Δ_z = 0.53, δ = 0.17≤max δ = 0.32, a = 0.80≤max a = 1.60, r = 1.0000≥min r = 0.9993).

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

UDC 311.16;547.447

THE QUANTITATIVE RELATIONSHIPS OF THE PARTITION COEFFICIENTS CALCULATED IN THE SERIES OF N-R-AMINE FUNCTIONAL DERIVATIVES

M.Yu.Golik, O.S.Kryskiv, A.M.Komissarenko, O.V.Kolisnyk, K.I.Dudka

National University of Pharmacy

Key words: partition coefficient; calculation methods; correlation; N-R-amine derivatives

Using on-line services and the ChemBioOffice2014 software package the values of the partition coefficients of some N-R-amine derivatives have been calculated. To identify the quantitative relationships and select the optimal algorithm of calculations the correlation and regression analysis of the values obtained has been conducted. Statistically significant values of correlation for the partition coefficients calculated have been determined. It has been shown that it is advisable to use the value AlogPs for further application.

Lipophilicity is one of the criteria for assessing the similarity of synthetic substances and drugs, and it affects the biological activity of substances [9, 13, 14]. The partition coefficient of *n*-octanol-water (logP) characterizes physicochemical properties of substances in solutions and is used to simulate their behaviour in the body [3, 14]. Distribution and redistribution of substances between lipophilic (fatty) and aqueous phases are part of biological processes, in particular transmembrane transport through lipid layers [11]. Hydrophobicity plays an important role in determining distribution of a substance in the body after absorption, its metabolic rate and elimination. The hydrophobic effect is essential for binding of drugs with their receptors [13].

The ability to predict biological properties of compounds through their lipophilicity allows to optimize the search for new drugs and is often included in equations for calculations of the quantitative structure – activity relationships (QSAR) [16].

The value logP is experimentally determined by the classical method of measuring distribution of organic compounds between the non-polar phase and water [14] using chromatographic [18], electrochemical [23] and other methods.

However, experimental methods to determine logP are time-consuming and expensive, and the values obtained often differ due to the influence of many factors. Therefore, today a significant number of algorithms for theoretical calculations of values logP implemented using the appropriate software [5, 6, 8, 17, 24, 25].

The values of the partition coefficients of *n*-octanol-water experimentally obtained with the values theoretically calculated by different algorithms (AlogPs, IAlogP, ClogP, miLogP, $logP_{KowWIN}$, xlogP3, etc.) were compared for 193 substances [19] and more than 96 000 compounds (including databases of Nykomed and Pfizer) [15]. In both studies the experimental data better correlate with the methods of AlogPs, xlogP3, $logP_{KowWIN}$.

Attempts to achieve proportionality of results of the theoretical calculation methods of prediction and the experimental determination of lipophilicity of organic compounds are still relevant in modern scientific studies [1, 16, 22].

The aim of this work is to determine the mutual correlation of the values of the partition coefficients in the series of some functional derivatives of N-R-amines (**1-21**) calculated by different algorithms.

Materials and Methods

Taking into account the data [15, 19] we used the available free on-line methods xlogP3 [25], AlogPs [6] for calculations of the partition coefficients, and the ChemBioOffice2014 software package, in particular ChemBioDrawUltra 14.0 (CBDU14) and ChemBio3DUltra 14.0 (CB3DU14) [7], for calculations of logP and ClogP (Table).

In total, the statistical sample included the comparison of 6 values of the partition coefficients calculated for 21 compounds. During the statistical processing of the research results when analysing the sample with the length of 21 cases the values of the Pearson correlation coefficient, which is more than 0.40 ($p \leq 0.05$), is considered to be statistically significant [2].

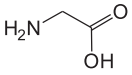
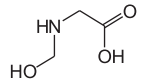
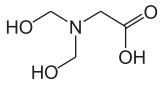
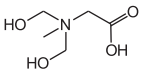
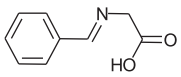
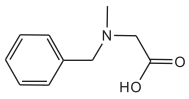
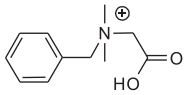
Calculations of correlations of the values of the partition coefficients calculated for compounds **1-21** were performed using the STATISTIKA 8 software [4, 21]. According to the requirements of mathematical statistics the correlation coefficient indicates the close relationship between the values: at values less than 0.3 – the relationship is absent, in the range of 0.3-0.7 it is medium, more than 0.7 – it is strong [10, 20].

Results and Discussion

As can be seen from Table, almost all compounds are characterized by negative values of the partition coefficients; probably it is due to the presence of the polar moiety – substituted Nitrogen atom in their structure. Increase of numeric values, and hence lipophilicity, is observed in the case of increase in the number of non-polar substituents (alkyl substituents or the phenyl nucleus in compounds **19-20**). Glycine (12), for which the calculated values (excluding logP) agree with the experimental ones [12], and its alkyl substituents (**13, 16**) are characterized by the maximum values of hydrophilicity. It can be also explained by the considerations previously mentioned.

Table

Structures and the values logP calculated of compounds **1-21**

No.	Structure	AlogPs	xlogP3	logP CBDU14	logP CB3DU14	ClogP CBDU14	ClogP CB3DU14
1	NH_3		-0.73			-1.2	-1.2
2	CH_3NH_2	-1.06	-0.71	-0.65	-0.57	-0.664	-0.664
3	$CH_3CH_2NH_2$	-0.2	-0.35	-0.32	-0.32	-0.135	-0.135
4	$HOCH_2CH_2NH_2$	-1.53	-1.31	-1.17	-1.31	-1.295	-1.295
5	$(CH_3)_2NH_2$	-0.53	-0.20	-0.13	-0.134	-0.518	-0.518
6	$(CH_3CH_2)_2NH$	0.76	0.58	0.54	0.50	0.54	0.54
7	$(HOCH_2CH_2)_2NH$	-1.41	-1.43	-1.17	-1.43	-1.463	-1.463
8	$(CH_3)_3N$	-0.14	0.26	0.24	0.27	0.018	0.018
9	$(CH_3CH_2)_3N$	1.57	1.45	1.26	1.44	1.605	1.605
10	$(HOCH_2CH_2)_3N$	-1.38	-1.00	-1.31	-2.218	-1.228	-1.228
11	$(CH_3)_4N^+$	-1.59	0.28			-4.856	-4.856
12		-3.34	-3.21	-1.39	-3.0	-3.210	-3.210
13		-3.24	-3.73	-0.97	-2.296	-2.811	-2.811
14		-1.76	-3.60	-0.97	-3.293	-1.742	-1.742
15		-1.94	-1.30			-3.747	-3.747
16		-3.06	-2.78	-0.87	-1.275	-3.124	-3.124
17		-1.70	-2.91	-0.49	-0.6	-2.368	-2.368
18		-1.90	-0.13			-4.173	-4.173
19		1.39	1.26	1.5	1.5	0.224	0.224
20		-0.32	-0.93	1.24	1.24	-0.600	-0.600
21		-1.40	1.37			-1.755	-1.755

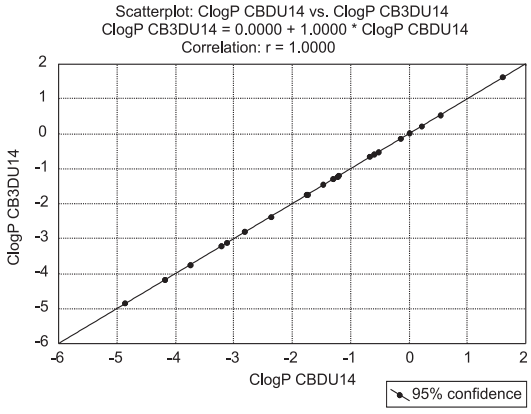


Fig. 1. Correlation of ClogP CBDU14 and ClogP CB3DU14.

Analysis of the results of statistical processing indicates that the coefficients of lipophilicity calculated by different algorithms for compounds (**1-21**) correlate (%) with each other in a different way and are statistically significant (Fig. 1-11).

It can be noted that the algorithms for calculating logP in ChemBioDrawUltra 14.0 and ChemBio3DUltra 14.0 do not allow to determine a numeric value for ionic compounds (**11, 18, 21**), and compounds that do not contain Carbon atoms (in our case NH_3 – compound **1**) (Table). The latter is observed for calculation of AlogPs. It should also be noted that the values ClogP calculated by Chem BioDrawUltra 14.0 and ChemBio3DUltra 14.0 are absolutely the same (r = 1.000) unlike the values logP similarly obtained where the correlation is slightly less (r = 0.91745) (Table, Fig. 1, 2).

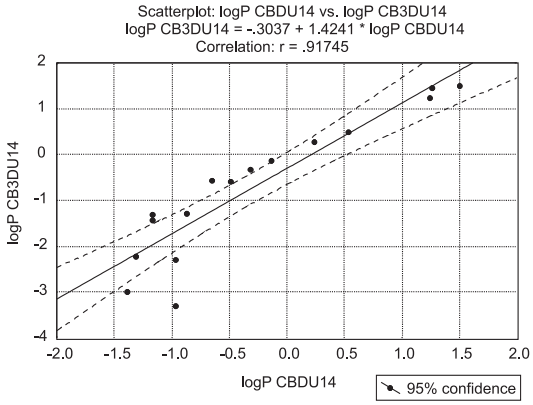


Fig. 2. Correlation of logP CBDU14 and logP CB3DU14.

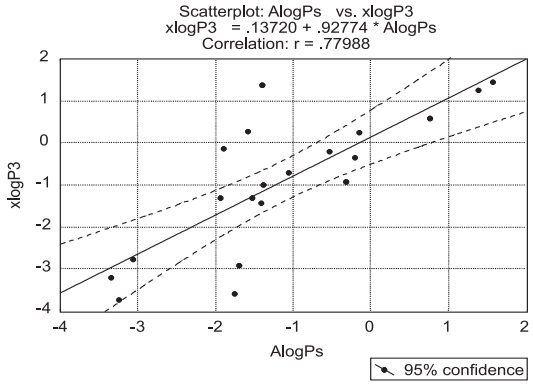


Fig. 3. Correlation of AlogPs and xlogP3.

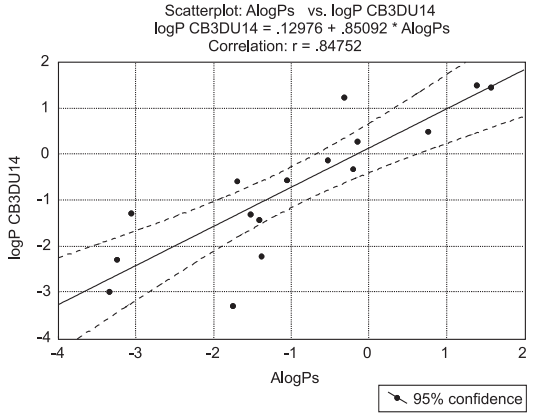


Fig. 5. Correlation of AlogPs and logP CB3DU14.

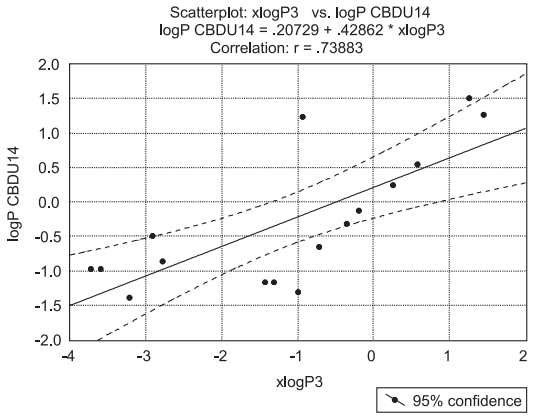


Fig. 7. Correlation of xlogP3 and logP CBDU14.

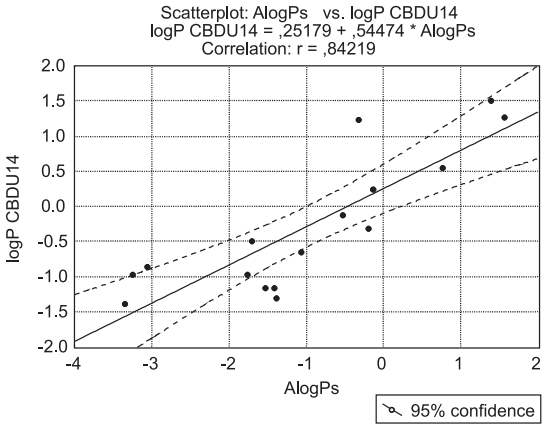


Fig. 4. Correlation of AlogPs and logP CBDU14.

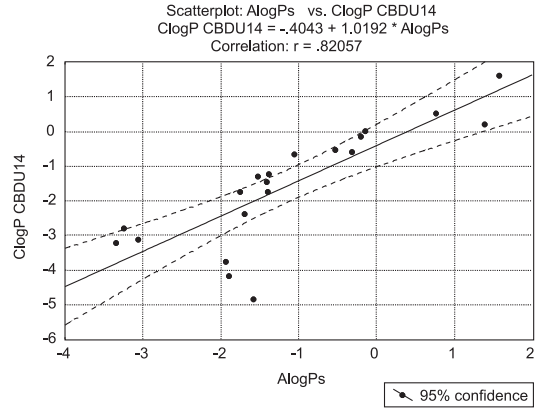


Fig. 6. Correlation of AlogPs and ClogP.

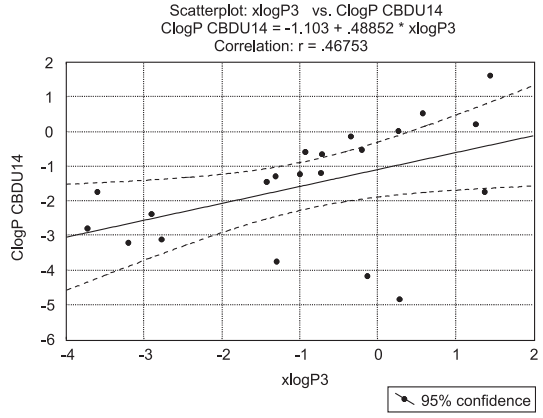


Fig. 8. Correlation of xlogP3 and ClogP.

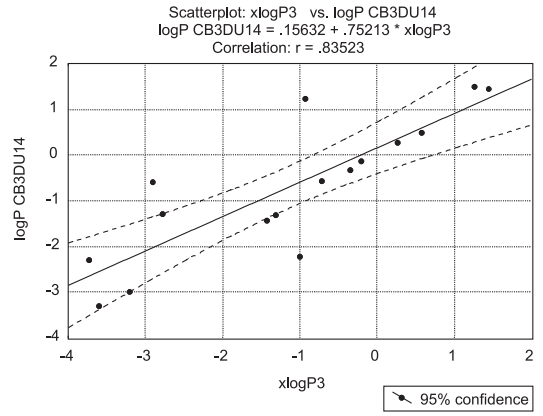


Fig. 9. Correlation of xlogP3 and logP CB3DU14.

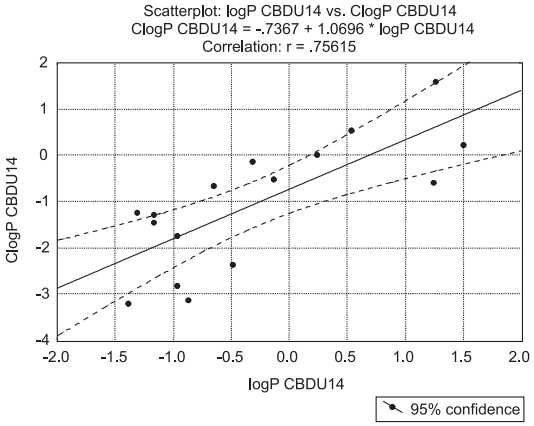


Fig. 10. Correlation of logP CBDU14 and ClogP.

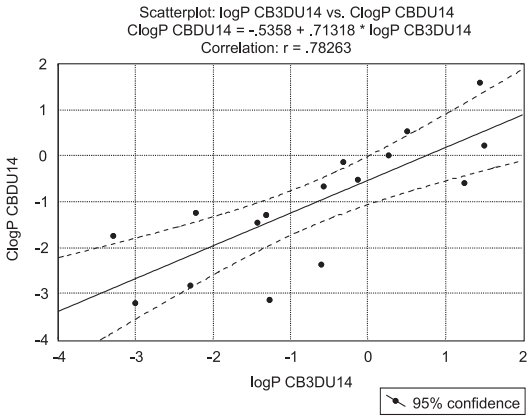


Fig. 11. Correlation of logP CB3DU14 and ClogP.

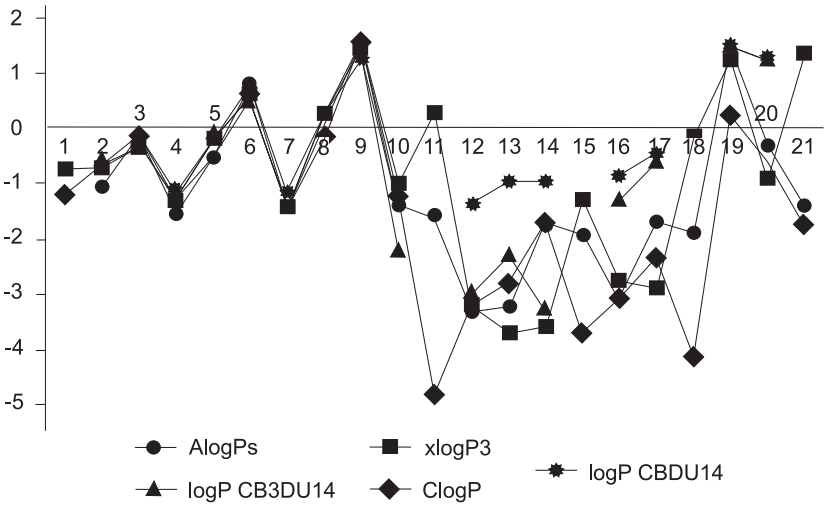


Fig. 12. The relationship of values of the partition coefficients calculated by different algorithms.

The maximum values of correlations that are consistent with the published data [15, 19] are observed in cases of AlogPs and logP CBDU14 (r = 0.84219), as well as AlogPs and logP CB3DU14 (r = 0.84752) (Fig. 4, 5, respectively). Somewhat smaller values are in the cases of xlogP3 and logP CB3DU14 (r = 0.83523) (Fig. 9), as well as AlogPs and ClogP (r = 0.82057) (Fig. 6). The minimum correlation was in the case of xlogP3 and ClogP (r = 0.46753) (Fig. 8). Other combinations occupied the intermediate values within the range of 73-78%: AlogPs and xlogP3 (r = 0.77988), xlogP3 and logP CBDU14 (r = 0.73883), logP CBDU14 and ClogP (r = 0.75615), logP CB3DU14 and ClogP (r = 0.78263) (Fig. 3, 7, 10, 11).

These combinations of the Pearson correlation coefficients and indicators of significance indicate the reliability of the graphs and equations shown in Fig. 1-11.

The position of the curve the values AlogPs theoretically calculated in relation to the results of other calculations of the partition coefficients (Fig. 12) may indicate the use of the optimally averaged algorithm of their determination.

Therefore, the results of our study allow to propose the values AlogPs obtained for further application when determining QSAR, as well as the degree of its manifestation among N-R-amine derivatives for planning a targeted search biologically active substances in this series.

CONCLUSIONS

1. Using on-line services and the ChemBioOffice2014 software package the values of the partition coefficients of some N-R-amine derivatives have been calculated.

2. To identify the quantitative relationships and select the optimal algorithm of calculations the correlation and regression analysis of the values obtained has been conducted.

3. Statistically significant values of correlation for the partition coefficients calculated have been determined. It has been shown that it is advisable to use the value AlogPs for further application.

REFERENCES

1. *Андреева Е.П., Раевский О.А. // Хим.-фарм. журн. – 2009. – Т. 43, №5. – С. 28-32.*
2. *Гублер Е.В., Генкин А.А. Применение непараметрических критериев статистики в медико-биологических исследованиях. – Л.: Медицина, 1973. – 141 с.*
3. *Раевский О.А. // Рос. хим. журн. – 2006. – №2. – С. 97-107.*
4. *Халафян А.А. Статистический анализ данных. STATISTICA 6: Учебник. – 3-е изд. – М.: Бином-Пресс, 2007. – 512 с.*

5. *ADMET and physchem predictions and related tools. [Electronic resource] (viewed on January 05, 2016). – Mode of access: http://www.vls3d.com/links/chemoinformatics/admet/admet-and-physchem-predictions-and-related-tools/ – Title from the screen.*
6. *AlogPs 2.1. [Electronic resource] (viewed on January 05, 2016). – Mode of access: http://146.107.217.178/lab/alogps/ – Title from the screen.*
7. *ChemOffice Professional [Electronic resource] (viewed on January 05, 2016). – Mode of access: https://www.cambridgesoft.com/Ensemble_for_Chemistry/ChemOffice/ChemOfficeProfessional/ – Title from the screen.*
8. *ClogP 4.0. [Electronic resource] (viewed on January 05, 2016). – Mode of access: http://www.biobyte.com/bb/prod/clogp40.html/ – Title from the screen.*
9. *Edwards M.P., Price D.A. // Annual Reports in Medicinal Chemistry. – 2010. – №45. – P. 381-391. Doi:10.1016/S0065-7743(10)45023-X. ISBN 978-0-12-380902-5.*
10. *Fujita T. // QSAR. – 1997. – Vol. 16. – P. 107-112.*
11. *Gertz M., Kilford P.J., Houston J.B. // Metabolism and Disposition. – 2008. – Vol. 36, №3. – P. 535-542.*
12. *Hansch C., Leo A., Hoekman D.H. Exploring QSAR: Hydrophobic, Electronic and Steric Constants, American Chemical Society, Washington DC, 1995.*
13. *Leeson P.D., Springthorpe B. // Nat. Rev. Drug Discov. – 2007. – №6. – P. 881-890. Doi:10.1038/nrd2445. PMID 17971784.*
14. *Lipinski C.A., Lombardo F., Dominy B.W. et al. // Adv. Drug. Del. Rev. – 1997. – №23. – P. 3-25.*
15. *Mannhold R., Poda G., Ostermann C. // J. of Pharmac. Sci. – 2009. – Vol. 98, №3. – P. 861-893.*
16. *Mannhold R., Rekker R.F. // Perspectives in Drug Discovery and Design. – 2000. – Vol. 18, Issue 1. – P. 1-18.*
17. *miLogP2.2, Molinspiration property calculation service FAQ. [Electronic resource] (viewed on January 05, 2016). – Mode of access: http://www.molinspiration.com/services/logp.html/ – Title from the screen.*
18. *Odović J., Trbojević-Stanković J. // Hem. Ind. – 2009. – №63. – P. 33-37.*
19. *Pyka A., Babuska M., Zachariasz M. // Acta Poloniae Pharmaceutica – Drug Res. – 2006. – Vol. 63, №3. – P. 159-167.*
20. *Rivere P. // Courrier des statistique. – Paris: INSEE. – 1999. – №90. – P. 47-58.*
21. *Statistica. [Electronic resource] (viewed on January 05, 2016). – Mode of access: http://www.statsoft.com/Products/STATISTICA-Features/ – Title from the screen.*
22. *Thompson S.J., Hattotuwagama C.K., Holliday J.D. et al. // Bioinformation. – 2006. – №1. – P. 237-241.*
23. *Ulmeanu S.M., Jensen H., Bouchard G. // Pharm. Res. – 2003. – №20. – P. 1317-1322.*
24. *VCCLAB, Virtual Computational Chemistry Laboratory. [Electronic resource] (viewed on January 05, 2016). – Mode of access: http://www.vcclab.org, 2005/ – Title from the screen.*
25. *xlogP3. [Electronic resource] (viewed on January 05, 2016). – Mode of access: http://www.sioc-ccbg.ac.cn/?p=42&software=xlogp3/ – Title from the screen.*

КІЛЬКІСНІ СПІВВІДНОШЕННЯ РОЗРАХОВАНИХ КОЕФІЦІЄНТІВ РОЗПОДІЛУ У РЯДУ ФУНКЦІОНАЛЬНИХ ПОХІДНИХ N-R-АМІНІВ

М.Ю.Голік, О.С.Криськів, А.М.Комісаренко, О.В.Колісник, К.І.Дудка

Ключові слова: *коефіцієнт розподілу; розрахункові методи; кореляція; N-R-амінопохідні*

З використанням он-лайн сервісів та програмного пакету ChemBioOffice2014 розраховані значення коефіцієнтів розподілу деяких N-R-амінопохідних та з метою виявлення кількісних співвідношень і вибору оптимального алгоритму розрахунків проведено кореляційно-регресійний аналіз одержаних значень. Встановлені статистично достовірні значення кореляції розрахованих коефіцієнтів розподілу та показано, що для подальшого використання доцільно використовувати значення AlogPs.

КОЛИЧЕСТВЕННЫЕ СООТНОШЕНИЯ РАССЧИТАННЫХ КОЭФФИЦИЕНТОВ РАСПРЕДЕЛЕНИЯ В РЯДУ ФУНКЦИОНАЛЬНЫХ ПРОИЗВОДНЫХ N-R-АМИНОВ

Н.Ю.Голик, О.С.Крыськив, А.Н.Комиссаренко, Е.В.Колесник, К.И.Дудка

Ключевые слова: *коэффициент распределения; расчетные методы; корреляция; N-R-аминопроизводные*

При использовании он-лайн сервисов и программного пакета ChemBioOffice2014 рассчитаны значения коэффициентов распределения некоторых N-R-аминопроизводных и с целью выявления количественных соотношений и выбора оптимального алгоритма расчетов проведен корреляционно-регрессионный анализ полученных значений. Установлены статистически достоверные значения корреляции рассчитанных коэффициентов распределения и показано, что для дальнейшего использования целесообразно использовать значение AlogPs.

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

UDC 547.398.1:547.461.3

THE SYNTHESIS AND PHYSICOCHEMICAL PROPERTIES OF NEW DERIVATIVES OF 5-R-PHENYLAMINO-2-MERCAPTO-1,3,4-THIADIAZOLE

I.V.Sych, L.O.Perekhoda, Z.G.Ieromina, L.O.Grinevich, N.P.Kobzar, I.V.Drapak

National University of Pharmacy

Danylo Halytsky Lviv National Medical University

Key words: synthesis; 1,3,4-thiadiazole;^{1}H NMR-spectroscopy

With the aim of obtaining new biologically active substances the synthesis of amides of 5-R-phenylamino-1,3,4-thiadiazole-2-yl-thioacetate acid and 5-phenylamino-1,3,4-thiadiazole-2-yl-thio-1-phenon has been conducted. As initial substances 5-R-phenylamino-2-mercapto-1,3,4-thiadiazoles, the corresponding analides of chloracetate acid and chloracetate phenon were used. The reaction of alkylation was conducted in the ethanol medium in the presence of potassium hydroxide. The structure of the compounds synthesized has been confirmed by the method of ^{1}H NMR-spectroscopy. The physicochemical properties and the pharmacological potential of the substances synthesized are discussed. According to the results of calculation methods of prediction of the biological activity (PASSOnline) the probable types of the biological activity of the substances synthesized have been estimated. In compliance with the data of the PASSOnline computer-based prognosis the antineoplastic action (STAT inhibitor of transcription factors) is principally inherent for most compounds of this class.

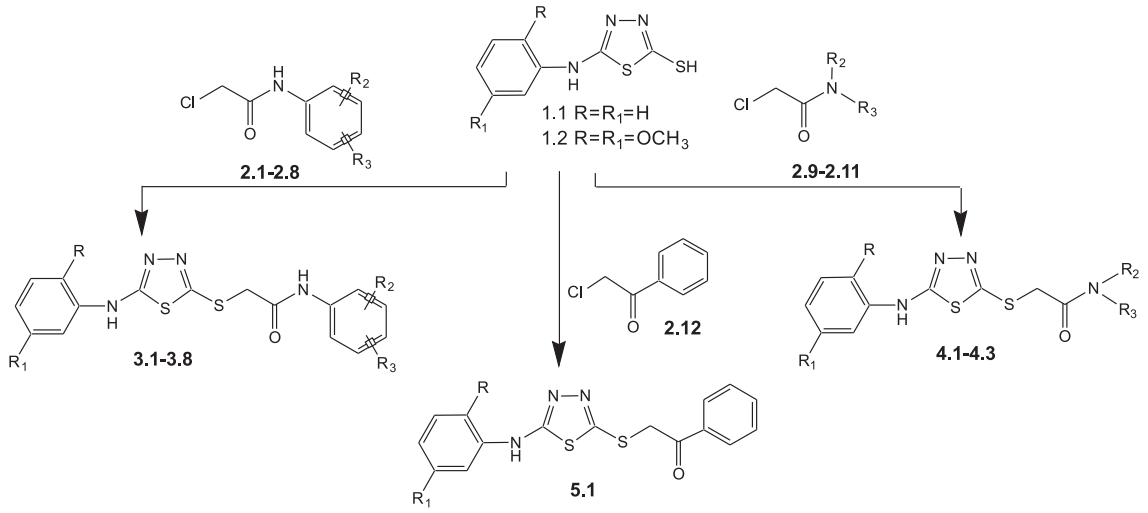
In the current realities the healthcare requires development and introduction of original medicines into production along with manufacture of generic drugs – only this way can raise the pharmaceutical industry of Ukraine to the higher level of quality and to rank it together with the leading European states. In this regard, creation of new effective medicines is a rather topical issue of modern pharmacy. Recently, along with the high pharmacological activity, the simplicity of synthetic methods and availability of the chemical raw material, which provide a relatively law prime cost of finished products, become the most important parameters for the choice of the research objects. With the aim of searching for new biologically active substances we paid attention to the derivatives of 1,3,4-thiadizole, which had already shown themselves as high-efficient bioactive substances exhibiting various types of the pharmacological action [2, 3, 5, 6, 8, 10, 11]. Currently, data on promising applications of derivatives of 1,3,4-thiadiazole for treating cancer appear in scientific literature more often [5, 10-11]. At the moment, the fundamental research of new derivatives are conducted on the basis of this heterocycle as potential antimicrobial agents [3, 6] and anticonvulsants [2, 8]. Thus, the further search of new bioactive substances among the derivatives of 1,3,4-thiadizole is one of the promising directions.

The aim of this work was to extend the range of potential bioactive substances on the basis of 5-R-phenylamino-2-mercapto-1,3,4-thiadiazoles. The synthesis of derivatives of 5-R-phenylamino-2-mercapto-1,3,4-thiadiazoles ***1.1-1.2*** was carried out by the interaction of carbonic disulphide and R-phenylthiosemicarbazide in the dimethylformamide medium at the temperature of 75°C with satisfactory yields (78-80%) by the known method [8]. The structure of the compounds obtained was confirmed by modern physical and chemical methods. Formation of the thiadiazole cycle was proven by the absence of NH-NH signal in the ^{1}H NMR-spectra of the compounds synthesized at δ 10.60-10.64 ppm and the presence of a singlet of the mercapto group at approximately 3.40 ppm [1, 9].

The target compounds – anilides of 5-(2,5-dimethoxy) phenylamino-1,3,4-thiadiazole-2-yl-thioacetate acid ***3.1-3.8*** and amides of 5-phenylamino-1,3,4- thiadiazole-2-yl-thioacetate acid ***4.1-4.3*** were obtained by S-alkylation with anilidies ***2.1-2.8*** and alkylamides ***2.9-2.11*** of chloracetate acid.

5-R-phenylamino-2-mercapto-1,3,4-thiadiazoles ***1.1-1.2*** were obtained in ethanol in the presence of the equimolar quantity of potassium hydroxide according to the Scheme given below.

The research results have proven that application of the method allows obtaining the target products with satisfactory yields and sufficient purity (Tab. 1). All compounds obtained after crystallization from ethanol are white crystalline substances with distinct melting points, soluble in most organic solvents (Tab. 1). To study the course of the alkylation reaction when using various alkali reagents the amides of chloracetate acid ***2.1-2.11*** were substituted by chloracetophenon ***2.12.*** (Scheme). The change of the alkylating agent did not affect substantially the yield of the finish product (5-phenylamino-1,3,4-thiadiazole-2-yl-thio-1-phenon ***5.1*** was obtained with the yield of 78%). The structure of the compounds obtained was confirmed by physical and spectral methods. The ^{1}H NMR-spectroscopy was performed, and the melting points of the compounds synthesized were determined. The composition of elements experimentally found corresponds exactly to the structures proposed.



Scheme

Table 1

Physical and chemical properties of the compounds synthesized

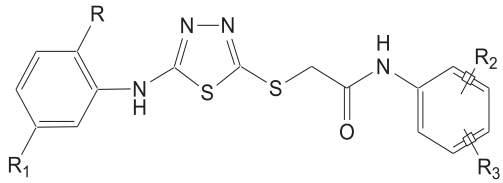
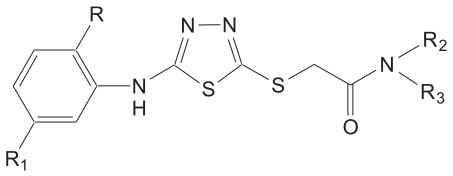
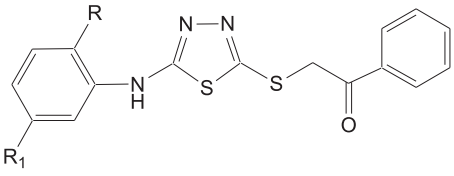
Compound	R	R_1	R_2	R_3	Yield, %	M. p., °C	Chemical formula	N, %	
								counted	founded
									
3.1	OCH_3	OCH_3	3 – CH_3	6 – CH_3	72	112-116	$C_{22}H_{22}N_4O_3S_2$	13,01	13,03
3.2	OCH_3	OCH_3	4 – $COOCH_3$	H	70	128-130	$C_{20}H_{20}N_4O_5S_2$	12,17	12,18
3.3	OCH_3	OCH_3	3 – Cl	4 – Cl	69	136-138	$C_{18}H_{16}Cl_2N_4O_3S_2$	11,89	11,91
3.4	OCH_3	OCH_3	2 – $COOCH_2$-CH_3	H	73	128-132	$C_{21}H_{22}N_4O_5S_2$	11,81	11,82
3.5	OCH_3	OCH_3	NHR = naphthyl		69	138-140	$C_{22}H_{20}N_4O_3S_2$	12,38	12,39
3.6	OCH_3	OCH_3	Bn	H	73	130-132	$C_{17}H_{16}N_4O_3S_2$	14,42	14,43
3.7	OCH_3	OCH_3	2 – CH_3	4 – CH_3	73	116-122	$C_{20}H_{22}N_4O_3S_2$	13,01	13,03
3.8	OCH_3	OCH_3	2 – Cl	3 – Cl	74	120-122	$C_{18}H_{16}Cl_2N_4O_3S_2$	11,89	11,90
									
4.1	H	H	$CH(CH_3)_2$	C_6H_5	79	160-162	$C_{19}H_{20}N_4OS_2$	14,57	14,58
4.2	H	H	H	3,6 – $OCH_3C_6H_4$	80	140-144	$C_{18}H_{18}N_4O_3S_2$	13,92	13,94
4.3	H	H	H	Bn	79	148-152	$C_{17}H_{16}N_4OS_2$	15,72	15,73
									
5.1	H	H	H	H	78	148-150	$C_{16}H_{13}N_3OS_2$	12,83	12,84

Table 2

Chemical shift (δ, ppm) of protons in 1H NMR spectra of the compounds synthesized

Compound	CONH (1H, s)	NH-R (1H, s)	2×OCH_3 (6H, s)	H-Ar	SCH_2 (2H, s)	Signals of protons of other functional groups
3.1	9,55	9,35	3,75	6,45, s, 1H; 6,80, d, 2H; 7,00, s, 1H; 7,40, s, 1H; 8,05, s, 1H.	4,05	2,30 (6H, s, 2×CH_3)
3.2	10,40	9,55	3,75	6,45-6,70, dd, 2H; 7,70, s, 1H; 7,90, s, 3H; 8,05, s, 1H.	4,45	2,50 (3H, s, CH_3)
3.3	10,40	9,55	3,75	6,45, s, 1H; 6,80, s, 1H; 7,35-7,50, dd, 2H; 7,90-8,05, dd, 2H.	4,05	–
3.4	9,55	9,35	3,75	6,45, s, 1H; 6,80-7,00, m, 4H; 8,05, s, 1H; 8,15, s, 1H.	4,10	4,05 (2H, s, CH_2) 1,40 (3H, s, CH_3)
3.5	10,10	9,60	3,75	6,45, s, 1H; 6,85, s, 1H; 7,40-7,50, m, 3H; 7,85, s, 2H 8,15, s, 2H.	4,20	–
3.6	9,05	8,45	3,75	6,45-6,85, dd, 2H; 7,15-7,30, m, 5H; 8,00, s, 1H.	4,30	3,80 (2H, s, CH_2)
3.7	9,55	9,35	3,75	6,45, d, 1H; 6,90-7,00, m, 4H; 7,30-7,40, m, 1H.	4,05	2,90 (6H, s, 2×CH_3)
3.8	9,58	9,45	3,75	6,45-6,80, dd, 2H; 7,60, s, 1H; 7,90, s, 3H; 8,10, s, 1H.	4,10	–
4.1	–	10,15	–	4,80, d, 1H; 6,90, d, 1H; 7,20-7,35, d, 4H; 7,40-7,55, d, 5H.	3,70	4,80 (1H, s, CH) 1,50 (6H, s, 2×CH_3)
4.2	10,39	9,49	3,7	6,55, d, 1H; 6,90-7,00, d, 2H; 7,30, d, 2H; 7,55, d, 2H; 7,75, s, 1H.	4,14	–
4.3	10,29	8,65	–	6,90, d, 1H; 7,19-7,35, m, 5H; 7,55, d, 2H.	4,30	3,92 (2H, s, CH_2)
5.1	10,23	–	–	6,90, d, 1H; 7,30, m, 2H; 7,50-7,70, m, 5H; 8,05, d, 2H.	4,88	–

Compounds **3.1-3.8, 4.2-4.3, 5.1** that contain the amide group in ^{1}H NMR-spectra have a general appropriate singlet signal of the NH-amide proton in the weak field in the interval of δ 9.05-10.39 ppm. The methylene group is located near a strong acceptor represented on the ^{1}H NMR-spectra by the shift of the singlet of the methylene group protons to the weak field (3.70-4.88 ppm). The spectra of compounds **3.1-3.8** have general signals of the aromatic protons of the 2,5-dimethoxyaminophenylic fragment registered in the range of δ 6.45-8.15 ppm.

Table 3

The PASS-prognosis of the biological activity for 5-substituted derivatives of 2-mercapto-1,3,4-thiadiazole

Compound	STAT3 inhibitor of transcription factor	STAT inhibitor of transcription factor	Inhibitor of cytidine-desaminase	Anta-gonist Mcl-1	Inhibitor of transcription factor	Inhibitor of calpain	Inhibitor of Cl-transporting of ATphase	Inhibitor of acro-cylindro-pepsin	Inhibitor of sugar-pepsin
3.1	0,725	0,705	0,673	0,579	0,547	0,541	–	–	–
3.2	0,734	0,713	0,687	0,588	0,559	–	–	–	–
3.3	0,669	0,635	0,605	0,582	–	0,542	–	–	–
3.4	0,677	0,648	0,649	0,529	0,584	0,552	–	–	–
3.5	0,581	0,563	0,569	0,587	–	0,523	–	–	–
3.6	0,604	0,580	–	–	–	0,546	–	–	–
3.7	0,713	0,689	0,658	0,564	0,536	0,531	–	–	–
3.8	0,608	0,577	0,567	0,582	–	0,506	–	–	–
4.1	–	–	–	0,545	–	–	0,586	0,525	0,525
4.2	0,732	0,780	0,598	0,643	0,597	0,517	–	–	–
4.3	0,539	0,581	–	0,571	–	0,533	0,541	–	–
5.1	0,568	0,607	–	0,698	0,521	–	0,724	–	–

The difference of ^{1}H NMR-spectra of the structures obtained from spectra of the initial compounds is in disappearance of a one-proton singlet of the mercapto group at δ 3.40 ppm and appearance of compounds of the CONH-group singlet on the spectra (Tab. 2).

According to the results of calculation methods of prediction of the biological activity (PASS) the probable types of the biological activity of the substances synthesized have been estimated. In compliance with the data of the PASS computer-based prognosis the antineoplastic action (STAT inhibitor of transcription factors) is principally inherent for most compounds of this class. In this series of the compounds synthesized the index of probability of STAT inhibition of transcription factors is in the range from 0.568 to 0.725, and it indicates the prospects of research for the antitumor activity (Tab. 3) [4, 7].

Experimental Part

Melting points were determined by the capillary method on the Kofler unit. The elemental analysis of the nitrogen content was conducted by the Dumas method.

^{1}H NMR-spectra were registered on a Varian Mercury device at the frequency of 200 MHz, the solvent – DMSO-d_6, the internal standard – tetramethylsilane (TMS). The chemical shifts are presented in the scale δ (ppm).

5-Phenylamino-2-mercapto-1,3,4-thiadiazole 1.1. Dissolve 104.65 g of 5-phenylthiosemicarbazide in dimethylformamide in the presence of methyl(methoxy) ammonium salt of 2,5-dimercaptothiadiazole at the temperature of 75°C. Then add 45 of carbon disulphide. Recrystallize from ethanol.Yield – 83.72 g (80%); M. p. – 180-182°C. Chemical formula: $C_8H_7N_3S_2$; ^{1}H NMR, DMSO-d_6, d, ppm: 3.40, 1H, s, (SH); 7.05-7.64, 5H, m (Ar-H); found N % 20.09;calculated N % 20.08.

Compound ***1.2*** was obtained by the similar method.

3,6-Dimethylanilide of 5-(2',5'-dimethoxy)phenylamino-1,3,4-thiadiazole-2-yl-thioacetate acid 3.1. To solution of 2.7 g (0.01 Mole) 5-(2',5'-dimethoxyphenilamino)-2-mercapto-1,3,4-thiadiazole in 50 ml of ethanol add 0.015 Mole (0.84 g) of potassium hydroxide while stirring. Then add 2.3 g (0.01 Mole) of 3,6-dimethylanilide of chloracetate acid to 30 ml of ethanol. Boil under reflux the reaction mixture for an hour, after that evaporate to dryness. Triturate a dry extract in 150 ml of water, filter the precipitate, and dry. Recrystallize from ethanol.

Anilides ***3.2-3.8***, amides ***4.1-4.3***, 5-phenylamino-1,3,4-thiadiazole-2-yl-thio-1-phenon ***5.1*** were obtained by the similar method.

CONCLUSIONS

The synthesis of 5-R-phenylamino-2-mercapto-1,3,4-thiadiazoles, amides of 5-R-phenylamino-1,3,4-thiadiazole-2-yl-thioacetate acid and 5-phenylamino-1,3,4-thiadiazole-2-yl-thio-1-phenon has been carried out. The chemical structure of the compounds synthesized has been proven by ^{1}H NMR-spectroscopy.

REFERENCES

1. *Breitmaier E. Structure elucidation by NMR in organic chemistry. – John Wiley @ Sons Ltd, 2002. – 258 p.*
2. *Chhajed M.R., Shrivastava A.K., Tailec V.S. // Macroheterocycles. − 2013. − Vol. 6, №2. − P. 199-209.*
3. *Hussain S., Sharma J., Amir M. // E-Journal of Chem. − 2008. − Vol. 5, №4. − P. 963-968.*
4. *ibmc.msk.ru/PASS/PASSASS.html*
5. *Mullick P., Khan S.A., Verma S., Alam O. // Bul. of the Korean Chem. Soc. – 2011. – Vol. 32, №3. − P. 1011-1016.*
6. *Onkol T., Doruer D.S., Uzun L. et al. // J. of Enzyme Inhibition and Med. Chem. − 2008. − Vol. 23, №2. − P. 277-284.*

7. *Poroikov V., Akimov D., Shabelnikova E. et al. // SAR and QSAR in Environmental Res. – 2001. – Vol. 12, №4. – P. 327-344.*
8. *Rajak H., Aggarwal N., Kashaw S. et al. // J. of the Korean Chem. Soc. − 2010. − Vol. 54, №1. − P. 158-164.*
9. *Silverstein R.M. Spectrometric identification of organic compounds. – 6th ed. – NY: John Wiley & Sons, 2001. – 196 p.*
10. *Sun J, YangY., Li W. et al. // Bioorg. Med. Chem. Lett. − 2011. – Vol. 21, №20. – P. 6116-6121.*
11. *Zhao J., Xuan L., Zhao H. et al. // Chem. Res. in Chinese Universities. − 2014.− Vol. 30, Iss. 5. − P. 764-769.*

СИНТЕЗ ТА ФІЗИКО-ХІМІЧНІ ВЛАСТИВОСТІ НОВИХ ПОХІДНИХ 5-R-ФЕНІЛАМІНО-2-МЕРКАПТО-1,3,4-ТІАДІАЗОЛУ

І.В.Сич, Л.О.Перехода, З.Г.Єрьоміна, Л.О.Гріневич, Н.П.Кобзар, І.В.Драпак

Ключові слова**:** *синтез; 1,3,4-тіадіазол; ^{1}Н ЯМР-спектроскопія*

З метою одержання нових біологічно активних речовин здійснено синтез амідів 5-R-феніламіно-1,3,4-тіадіазол-2-іл-тіоацетатної кислоти та 5-феніламіно-1,3,4-тіадіазол-2-ілтіо-1-фенону. Як вихідні сполуки використовували 5-R-феніламіно-2-меркапто-1,3,4-тіадіазоли, відповідні аніліди хлорацетатної кислоти та хлорацетофенон. Реакція алкілування проведена в середовищі етанолу в присутності калію гідроксиду. Структура синтезованих сполук підтверджена методом ^{1}Н ЯМР-спектроскопії. Обговорені фізико-хімічні властивості та фармакологічний потенціал синтезованих речовин. За результатами розрахункових методів прогнозування біологічної активності (PASSOnline) були оцінені вірогідні види біологічної дії синтезованих речовин. У відповідності з даними комп'ютерного прогнозу PASSOnline більшості сполук цього класу насамперед притаманна протипухлинна дія (інгібітор STAT факторів транскрипції).

СИНТЕЗ И ФИЗИКО-ХИМИЧЕСКИЕ СВОЙСТВА НОВЫХ ПРОИЗВОДНЫХ 5-R-ФЕНИЛАМИНО-2-МЕРКАПТО-1,3,4-ТИАДИАЗОЛА

И.В.Сыч, Л.А.Перехода, З.Г.Еремина, Л.А.Гриневич, Н.П.Кобзарь, И.В.Драпак

Ключевые слова**:** *синтез; 1,3,4-тиадиазол; ^{1}Н ЯМР-спектроскопия*

С целью получения новых биологически активных веществ осуществлен синтез амидов 5-R-фениламино-1,3,4-тиадиазол-2-ил-тиоуксусной кислоты и 5-фениламино-1,3,4-тиадиазол-2-илтио-1-фенона. В качестве исходных веществ использовали 5-R-фениламино-2-меркапто-1,3,4-тиадиазолы, соответствующие анилиды хлоруксусной кислоты и хлорацетофенон. Реакция алкилирования проведена в среде этанола в присутствии калия гидроксида. Структура синтезированных соединений подтверждена методом ^{1}Н ЯМР-спектроскопии. Обсуждены физико-химические свойства и фармакологический потенциал синтезированных веществ. По результатам расчетных методов прогнозирования биологической активности (PASSOnline) были оценены возможные виды биологического действия синтезированных веществ. В соответствии с данными компьютерного прогнозирования PASSOnline для большинства соединений этого класса прежде всего характерно противоопухолевое действие (ингибитор STAT факторов транскрипции).

Recommended by Doctor of Pharmacy, professor I.M.Vladimirova

UDC 615.322: 615.213

A COMPARATIVE PHYTOCHEMICAL AND PHARMACOLOGICAL ANALYSIS OF THE EXTRACTS FROM LEAVES OF THE UKRAINIAN FLORA SHRUBS

N.A.Blyznyuk, Yu.S.Prokopenko, V.A.Georgiyants, V.V.Tsyvunin

National University of Pharmacy

Key words: phytochemical analysis; pharmacological analysis; Ukrainian flora; shrubs; extract

In the Ukrainian flora such plants as Forsythia europaea Deg. et Bald, Jasminum officinale L., Berberis thunbergii DC, Weigela hybrida Jaeg., Ligustrum vulgare L., and Corylus avellana L. hold a special place among shrubs. The aim of our research was to analyse the relationship between the chemical composition and the anticonvulsant activity of the extracts from leaves of the given shrubs. The highest content of flavonoids has been determined in aqueous extracts of Forsythia europaea (2.83%) and Corylus avellana (1.95%) leaves comparing to other extracts. The highest amount of polyphenols has been determined in Corylus avellana ethanol (96%) extract (1.31%) and Jasminum officinale ethanol (50%) extract (1.30%). The dry aqueous extract of Corylus avellana leaves has shown the most pronounced anticonvulsant activity. Dry extracts of Berberis thunbergii leaves, Weigela hybrida leaves, and Ligustrum vulgare leaves have not revealed a significant anticonvulsant activity. According to the strength of the effect the dry aqueous extract of Corylus avellana can be a promising substance for creating an original herbal remedy with anticonvulsant properties.

Garden plants, both ornamental and edible, are one of the largest groups of plants [13]. They are widely used in horticulture both for esthetic territory design and for protection from noise and dust, as well as for agricultural households.

In the Ukrainian flora such plants as *Forsythia europaea Deg. et Bald, Jasminum officinale L., Berberis thunbergii DC, Weigela hybrida Jaeg., Ligustrum vulgare L.,* and *Corylus avellana L.* hold a special place among shrubs, mostly due to their unintelligibility and effective appearance [8, 9]. Therefore, shrubs widespread in Ukraine are promising objects for scientific research since different types of phytochemical and pharmacological analysis will contribute to their future demand in pharmaceutical manufacturing.

Previously, different studies, particularly by scientists of the National University of Pharmacy, devoted to the phytochemical and pharmacological research of the Ukrainian flora shrubs were carried out. For instance, the membrane stabilizing, anti-inflammatory, anticoagulant, anti-ulcer, and strengthening blood vessel activities of *Corylus avellana L.* extracts were determined [3, 7]. The results of studying *Ligustrum vulgare L.* have shown the presence of different groups of compounds, as well as a pronounced anti-inflammatory and antimicrobial activity of the tincture from *Ligustrum vulgare L.* leaves [4].

While analysing publications devoted to the anticonvulsant activity of herbs some results concerning the study of the mechanisms of action for extracts from leaves, roots or buds of different either ornamental or edible shrubs, e. g. *Forsythia europaea Deg. et Bald*, *Jasminum grandiflorum* hydroalcoholic extract, *Berberis integerrima L.* (root), *Corylus avellana L.* (buds), etc., were found [10, 11, 12, 14, 16, 17]. Earlier, the chemical composition of leaves from the Ukrainian flora shrubs was analysed by the methods of absorption spectroscopy [1]. Nevertheless, during the analysis of the anticonvulsant activity of extracts of the plants mentioned the study of their chemical composition became relevant. Therefore, the aim of the present study was to analyse the relationship between the chemical composition of extracts from leaves of the Ukrainian flora shrubs and their anticonvulsant activity.

Materials and Methods

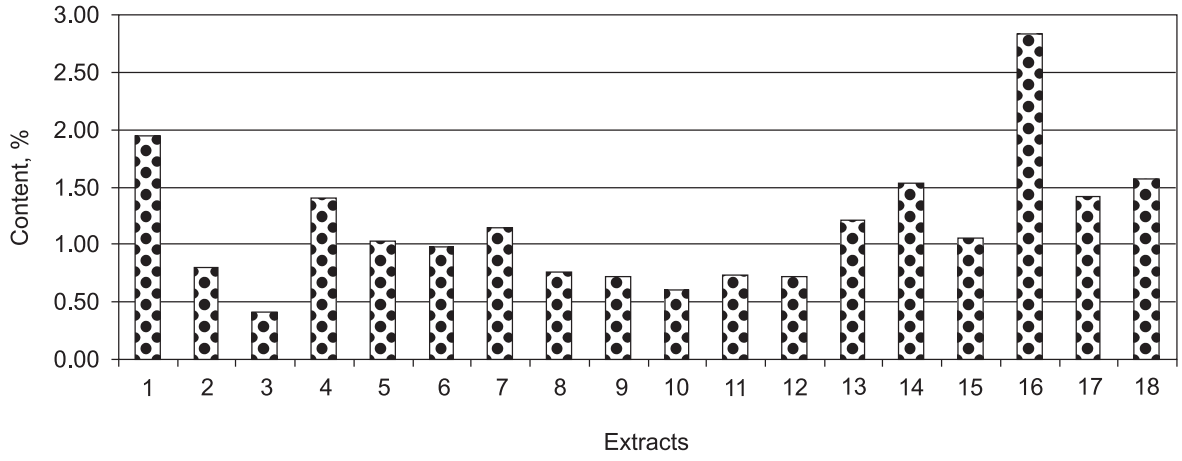
Reagents from Sigma-Aldrich (USA) and Merck (Germany) were used and prepared according to the requirements of the State Pharmacopoeia of Ukraine and European Pharmacopoeia.

Pentylenetetrazole was purchased from Sigma-Aldrich (USA).

Sodium Valproate was used in the form of syrup, 57.64 mg/1 ml (trade name Depakine, Sanofi-Aventis, France).

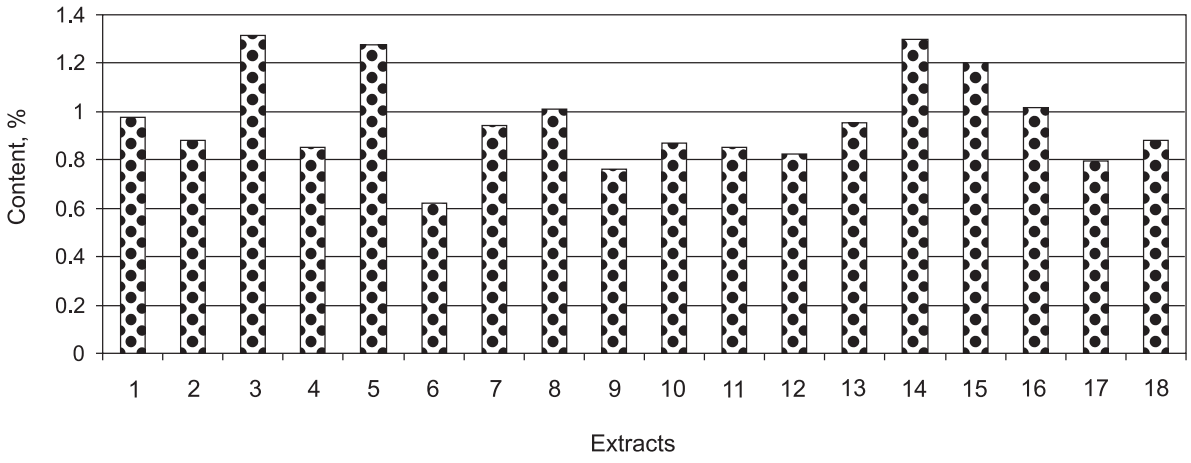
Leaves of *Forsythia europaea Deg. et Bald, Jasminum officinale L., Berberis thunbergii DC, Weigela hybrida Jaeg., Ligustrum vulgare L.,* and *Corylus avellana L.* were gathered during the flowering season (in full bloom) in Ukraine. The herbal material was cleaned and dried. After complete drying, the dry herbs were kept at room temperature. Then, herb samples were powdered and used for further research.

The extracts were prepared as follows. Place 100 g of the air-dried and powdered leaves into a percolator, and allow extraction to run using water, or 50% ethanol, or 95% ethanol as a solvent in the ratio of 1 to 20 at 80°C for 2 h. Then, filter the extracts and concentrate it in a vacuum-evaporation apparatus at 50-60°C and at 80-87 kPa to a thick consistency. Finally, dry each extract under



1 - C. avellana aqeous extract
2 - C. avellana ethanol (50%) extract
3 - C. avellana ethanol (96%) extract
4 - B. thunbergii aqeous extract
5 - B. thunbergii ethanol (50%) extract
6 - B. thunbergii ethanol (96%) extract
7 - L. vulgare aqeous extract
8 - L. vulgare ethanol (50%) extract
9 - L. vulgare ethanol (96%) extract
10 - W. hybrida aqeous extract
11 - W. hybrida ethanol (50%) extract
12 - W. hybrida ethanol (96%) extract
13 - J. officinale aqeous extract
14 - J. officinale ethanol (50%) extract
15 - J. officinale ethanol (96%) extract
16 - F. europaea aqeous extract
17 - F. europaea ethanol (50%) extract
18 - F. europaea ethanol (96%) extract

Fig. 1. The content of flavonoids in the extracts analysed.



1 - C. avellana aqeous extract
2 - C. avellana ethanol (50%) extract
3 - C. avellana ethanol (96%) extract
4 - B. thunbergii aqeous extract
5 - B. thunbergii ethanol (50%) extract
6 - B. thunbergii ethanol (96%) extract
7 - L. vulgare aqeous extract
8 - L. vulgare ethanol (50%) extract
9 - L. vulgare ethanol (96%) extract
10 - W. hybrida aqeous extract
11 - W. hybrida ethanol (50%) extract
12 - W. hybrida ethanol (96%) extract
13 - J. officinale aqeous extract
14 - J. officinale ethanol (50%) extract
15 - J. officinale ethanol (96%) extract
16 - F. europaea aqeous extract
17 - F. europaea ethanol (50%) extract
18 - F. europaea ethanol (96%) extract

Fig. 2. The content of polyphenols in the extracts analysed.

vacuum in the desiccators to yield a dry extract with a residual moisture content of 5%.

The assay for flavonoids was carried out according to the method developed [1, 2]. Flavonoid standard solutions of 100 μM were used.

The assay for polyphenols was carried out by the method of absorption spectrometry after adding the phosphorus molybdenum-tungsten reagent [2]. The reference solution of pyrogallol was used.

Adult male random-bred albino mice weighing 18-25 g were received from the vivarium of the Central Research Laboratory at the NUPh (Kharkiv, Ukraine). The animals were treated in accordance with Directive 2010/63/EU of the European Parliament and of the Council of September 22, 2010, on protection of animals used for scientific purposes. The animals were randomly divided into groups of 6-8 mice.

All the experimental protocols were approved by the Committee of Bioethics of the National University of Pharmacy.

All tested samples were dissolved (or suspended) in distilled water and administered into the stomach in the empiric dose of 100 mg/kg for 2 days [6]. The reference drug Sodium Valproate was introduced intragastrically in the dose of 300 mg/kg in a similar mode [5]. The control group was treated with distilled water. The seizure agent pentylenetetrazole was given subcutaneously (80 mg/kg) on the second day for 30 min after introduction of herbal samples. After that the animals were observed for 1 h [6].

The anticonvulsant activity was assessed using the following indicators: the latency period, the number of clonic-tonic convulsions per 1 mouse, % of mice with clonic and tonic convulsions, severity of seizures, duration of the convulsive period, the time of death, lethality.

The statistical analysis was carried out using the STATISTICA 8.0 software package. Differences between experimental groups were analysed using the Mann-Whitney U test and the Fisher angular transformation. The level of statistical significance was considered as $p < 0.05$.

Results and Discussion

The content of flavonoids and polyphenols in dry extracts from leaves of the Ukrainian flora shrubs was studied and recalculated. The results are summarized in Fig. 1 and in Fig. 2, respectively.

As shown in Fig. 1, the highest content of flavonoids was determined in aqueous extracts, in particular, in dry extracts of *Forsythia europaea* (2.83%) and *Corylus avellana* (1.95%). The lowest content of flavonoids was determined in the dry ethanol (96%) extract from *Corylus avellana* leaves (0.41%).

In general, the results presented in Fig. 2 show rather high amount of polyphenols in the extracts analysed. Thus, the highest amount of this group of compounds was determined in ethanol (96%) extract (1.31%) of *Corylus avellana*, ethanol (50%) extract (1.274%) of *Berberis thunbergii*, and ethanol (50%) extract (1.30%) of *Jasminum officinale*. The lowest content of polyphenols was determined in the dry ethanol (96%) extract from *Berberis thunbergii* leaves (0.623%) compared to the rest of the extracts analysed.

In mice subjected to Pentylenetetrazole-induced seizures [15] the dry aqueous extract of *Corylus avellana* leaves, as well as the reference drug Sodium Valproate, showed the most pronounced anticonvulsant activity, resulting in a significant increase in the latency period of the first seizure occurrence, reducing lethality and duration of the convulsive period in the group. Both dry ethanol (50%) and ethanol (96%) extracts from *Corylus avellana* leaves did not practically differ from each other in their anticonvulsant activity. In general, the anticonvulsant effect of *Corylus avellana* extracts increased with the ethanol concentration decrease.

Dry extracts of *Berberis thunbergii* leaves, *Weigela hybrida* leaves, and *Ligustrum vulgare* leaves did not show a significant anticonvulsant activity: duration of the convulsive period and severity of seizures increased, as well as high lethality levels were observed in the groups compared to the control animals.

Therefore, the dry aqueous extract of *Corylus avellana* leaves has shown high anticonvulsant properties, which probably depend on the synergism of the effect of biologically active compounds. According to the strength of the effect the dry aqueous extract of *Corylus avellana* can be a promising substance for creating an original herbal remedy with anticonvulsant properties.

Conclusions

The phytochemical analysis of dry extracts from the Ukrainian flora shrubs leaves has been carried out, and their anticonvulsant properties have been studied. The results have shown a high amount of polyphenols in the extracts analysed. The highest content of flavonoids has been determined in aqueous extracts, in particular, in *Forsythia europaea* (2.83%) and *Corylus avellana* (1.95%) dry extracts.

The dry aqueous extract of *Corylus avellana* leaves has shown high anticonvulsant properties compared to the other extracts analysed. At the same time, dry extracts of *Berberis thunbergii* leaves, *Weigela hybrida* leaves, and *Ligustrum vulgare* leaves have not revealed a significant anticonvulsant activity.

REFERENCES

1. *Близнюк Н.А., Прокопенко Ю.С., Георгіянц В.А. // Зб. наук. праць співроб. НМАПО ім. П.Л.Шупика. – 2015. – Вип. 24, №4. – С. 321-325.*
2. *Державна фармакопея України / Державне підприємство «Науково-експертний фармакопейний центр». – Доп. 4. – 1-е вид. – Х.: РІРЕГ, 2011. – 538 с.*
3. *Пат. на кор. модель 77795 (2006) Україна, МПК (2006) A 61 K 36/185, A 61 P 1/04, A 61 P 29/00, A 61 P 39/00. Спосіб отримання комплексу біологічно активних речовин з противиразковою, протизапальною та мембраностабілізуючою дією / О.П.Хворост. – Заявл.: 22.11.2004. Опубл.: 15.01.2007. – Бюл. №1.*

4. *Пат. на кор. модель 94496 (2014) Україна, МПК A 61 K 36/83. Лікарський засіб антимікробної дії / К.С.Мусієнко. – Заявл.: 19.06.2014. Опубл.: 10.11.2014. – Бюл. №4.*
5. *Цивунін В.В., Штриголь С.Ю., Прокопенко Ю.С. та ін. // Клінічна фармація. – 2012. – Т. 16, №4. – С. 47-50.*
6. *Цивунін В.В., Штриголь С.Ю., Прокопенко Ю.С. та ін. // УБФЖ. – 2014. – №3. – С. 45-49.*
7. *Юсіфова Д.Ю., Малоштан Л.М., Шаталова О.М. // УБФЖ. – 2014. – №6. – С. 47-50.*
8. *Сударікова Ю. Екзотичні дерева, кущі та ліани в ландшафтах України. – К.: Наш формат, 2011. – 336 с.*
9. *Bioecological justification assortment of shrubs for landscaping urban landscapes / A.Semenyutina, S.Kostyukov. – Montreal, 2013. – 164 p.*
10. *Gupta R. // Pharmacognos. Res. – 2013. – №5. – C. 286-290.*
11. *Hosseinzadeh H., Ramezani M., Shafaei H. // J. of Acupuncture and Meridian Studies. – 2013. – №6. – P. 12-17.*
12. *Ion T. // Farmacia. – 2009. – №57. – P. 141-156.*
13. *Planting: A New Perspective / N.Kingsbury, P.Oudorf. – Portland: Timber Press, 2013. – 280 p.*
14. *Sandeep P. // Pharmacol. On. News. – 2009. – №2. – P. 586-595.*
15. *Underlying Mechanisms of Epilepsy // Croatia: InTech Publisher, 2011. – P. 269-282.*
16. *Weici T., Gerhard E. Chinese Drugs of Plant Origin: Chemistry, Pharmacology, and Use in Traditional and Modern Medicine. – London: Springer, 1992. – 1056 p.*
17. *Wakeel O.K., Umukoro S., Kolawole O.T. et al. // Asian J. of Biomed. and Pharmac. Sci. – 2014. – №4. – P. 44-47.*

ПОРІВНЯЛЬНЕ ФІТОХІМІЧНЕ ТА ФАРМАКОЛОГІЧНЕ ВИВЧЕННЯ ЕКСТРАКТІВ ЛИСТЯ ЧАГАРНИКІВ ФЛОРИ УКРАЇНИ

Н.А.Близнюк, Ю.С.Прокопенко, В.А.Георгіянц, В.В.Цивунін

Ключові слова: *фітохімічний аналіз; фармакологічний аналіз; флора України; чагарники; екстракт*

У флорі України такі рослини, як Forsythia europaea Deg. et Bald, Jasminum officinale L., Berberis thunbergii DC, Weigela hybrida Jaeg., Ligustrum vulgare L. та Corylus avellana L. посідають особливе місце серед чагарників. Метою нашого дослідження було вивчення взаємозв'язку між хімічним складом та протисудомною активністю екстрактів з листя представлених рослин. У результаті проведеного аналізу було встановлено, що водні екстракти Forsythia europaea та Corylus avellana характеризуються найбільшим вмістом флавоноїдів у порівнянні з іншими екстрактами, що аналізувались. Сухий спиртовий (96%) екстракт Corylus avellana та сухий спиртовий (50%) екстракт Jasminum officinale характеризувались найвищим вмістом поліфенолів (1,314% та 1,301%, відповідно). Найбільш вираженим протисудомним ефектом характеризувався сухий водний екстракт листя Corylus avellana. Сухі екстракти листя Berberis thunbergii, Weigela hybrida і Ligustrum vulgare не проявили значних протисудомних властивостей. Отримані результати дозволяють охарактеризувати сухий водний екстракт Corylus avellana як перспективну субстанцію для розробки оригінального фітотерапевтичного засобу з протисудомною дією.

СРАВНИТЕЛЬНОЕ ФИТОХИМИЧЕСКОЕ И ФАРМАКОЛОГИЧЕСКОЕ ИЗУЧЕНИЕ ЭКСТРАКТОВ ЛИСТЬЕВ КУСТАРНИКОВ ФЛОРЫ УКРАИНЫ

Н.А.Близнюк, Ю.С.Прокопенко, В.А.Георгиянц, В.В.Цывунин

Ключевые слова*: фитохимический анализ; фармакологический анализ; флора Украины; кустарники; экстракт*

Во флоре Украины такие растения, как Forsythia europaea Deg. et Bald, Jasminum officinale L., Berberis thunbergii DC, Weigela hybrida Jaeg., Ligustrum vulgare L., и Corylus avellana L. занимают особое место среди кустарников. Целью нашего исследования было изучение взаимосвязи химического состава и противосудорожного действия экстрактов из листьев представленных растений. В результате проведенного анализа было обнаружено, что водные экстракты Forsythia europaea и Corylus avellana характеризуются наивысшим содержанием флавоноидов по сравнению с другими анализируемыми экстрактами. Сухой спиртовый (96%) экстракт Corylus avellana и сухой спиртовый (50%) экстракт Jasminum officinale характеризуются наивысшим содержанием полифенолов (1,31% и 1,30%, соответственно). Наиболее выраженным противосудорожным эффектом характеризуется сухой водный экстракт листьев Corylus avellana. Сухие экстракты листьев Berberis thunbergii, Weigela hybrida и Ligustrum vulgare не проявили выраженных противосудорожных свойств. Полученные результаты позволяют охарактеризовать сухой водный экстракт Corylus avellana как перспективную субстанцию для разработки оригинального фитотерапевтического средства с противосудорожным действием.

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

UDC 615.322:582.949.27:581.45:547.455:547.466:615.451.16

THE AMINO ACID AND MONOSACCHARIDE COMPOSITION OF A DRY EXTRACT FROM *SALVIA OFFICINALIS* LEAVES OBTAINED BY COMPLEX PROCESSING

G.V.Vovk, O.M.Koshovyi, A.M.Komissarenko

National University of Pharmacy

Key words: Salvia officinalis; leaves; dry extract; amino acid; monosaccharide; complex processing

The amino acid and monosaccharide composition of a dry extract from Salvia officinalis leaves obtained by complex processing has been studied. The preliminary determination of amino acids and monosaccharides has been performed by paper chromatography. The qualitative and quantitative analysis of free and bound amino acids and monosaccharides in the extract from Salvia officinalis leaves has been carried out using a high performance liquid chromatograph by Agilent Technologies company (model 1100) equipped with a flow vacuum degasser G1379A, a 4-channel pump of the low pressure gradient G13111A, an automatic injector G1313A, a column thermostat G13116A, diode array detector G1316A. As a result of the chromatographic studies of the amino acid and monosaccharide composition in the dry extract from Salvia officinalis leaves 10 free and 12 bound amino acids have been identified, six of which are indispensable – threonine, valine, isoleucine, leucine, phenylalanine and arginine, and 4 monosaccharides – glucose, galactose, rhamnose and arabinose. In the extract of Salvia officinalis leaves dominant substances are tyrosine, serine, glutamic and aspartic acid. In the dry extract of Salvia officinalis leaves the content of free (0.38%) and bound (0.43%) amino acids, monosaccharides (6.9%) increasing up to 11.2% after hydrolysis has been determined.

The problem of the rational use of the medicinal plant raw material (MPRM) by the pharmaceutical industry attracts more and more attention. Industrial waste products are tons of the extraction pomace containing a significant amount of biologically active substances and can be used to create new medicines. Despite the limited natural resources, the task of the modern pharmaceutical industry is development of methods for complex processing of MPRM allowing maximum use of its capabilities.

More than 40 drugs, which include biologically active substances (BAS) of *Salvia officinalis* leaves, have been registered at the market of Ukraine [3]. The composition of these drugs mainly includes the essential oil and tincture from the leaves of *Salvia officinalis*, i.e. the complex of lipophilic substances [3, 10, 11]. Previously the domestic pharmaceutical industry produced "Salvine" – 1% alcoholic solution of the acetone extract from *Salvia officinalis* leaves. Since later acetone was attributed to precursors, manufacturers discontinued the production of this drug, and "Salvine" disappeared from pharmacy shelves despite the efficiency of its use for the treatment of infectious and inflammatory diseases of the oral cavity. Acetone largely extracts substances of the terpenic origin (mono-, sesqui- and diterpenes), while the extraction pomace still contains more polar substances, in particular of the phenolic nature, amino acids and sugars [1, 4, 9]. In reference with the abovementioned facts, the method of complex processing of this raw material with obtaining the dry extract possessing the anti-inflammatory action has been developed [4, 5, 12].

Since amino acids and monosaccharides have a significant impact on bioavailability and the total therapeutic effect of the extract, the aim of our further studies is to investigate the amino acid and sugar composition of the dry extract from *Salvia officinalis* leaves obtained.

Materials and Methods

The object of the study was a dry aqueous extract of *Salvia officinalis* leaves obtained by complex processing [5]. The analysis of the extract was carried out according to the requirements of the SPhU [2, 6].

The preliminary chromatographic study of the qualitative composition of amino acids in a dry extract of *Salvia officinalis* leaves was conducted by the method of ascending chromatography on a "Filtrak No. 4" chromatography paper in the system of *n*-butanol – acetic acid – water (4:1:2) [1, 9]. For comparison the standard set of amino acids (TU 6-09-3147-83) in the concentration of 0.1% was used. Chromatograms were treated with 0.2% ninhydrin solution in acetone and dried in a drying cabinet at a temperature of 60-80°C. Amino acids were identified comparing with authentic samples of R_f values in parallel chromatographic procedure.

The qualitative composition and quantitative content of free and bound amino acids and monosaccharides in the extract from *Salvia officinalis* leaves was carried out using a high performance liquid chromatograph by Agilent Technologies company (model 1100) equipped with a flow vacuum degasser G1379A, a 4-channel pump of the low pressure gradient G13111A, an automatic injector G1313A, a column thermostat G13116A, diode array detector G1316A [7, 8].

For chromatography we used: column AA with the size of 200×2.1 mm and a guard column; as a mobile phase – solution A (20 mM of sodium acetate and 0.018% triethylamine adjusted to pH 7.2 with 1-2% acetic acid) with addition of 0.3% tetrahydrofuran, and solution B

Table

The amino acid composition of the dry extract from *Salvia officinalis* leaves

Amino acid	The content of amino acids (mg per 100 g of a dry extract)	
	Free	Bound
Aspartic acid	23.4	53.1
Glutamic acid	26.6	44.2
Serine	87.4	94.1
Valine	8.1	18.4
Arginine	0.0	7.1
Glycine	4.4	4.7
Threonine	9.2	21.1
Phenylalanine	18.0	23.5
Isoleucine	15.4	28.2
Leucine	20.1	21.7
Cysteine	0.0	51.9
Tyrosine	164.0	62.5

(40% CH_3CN, 40% MeOH and 20% of sodium acetate adjusted to pH 7.2 with 1-2% acetic acid); the volume flow rate – 0.450 ml/min; the compressibility of solution A – $50 \cdot 10^{-6}$ Bar, solution B – $115 \cdot 10^{-6}$ Bar; the column temperature – 40°C; detection was performed using an UV detector.

The sample preparation for studying the composition of free amino acids. In a 10 ml vial (A) add 0.3 ml of the extract. Then pour 3 ml of 0.1 N aqueous solution of hydrochloric acid containing 0.2% β-mercaptoethanol into the vial. Close the vial hermetically and place in an ultrasonic bath for 2 h at the temperature of 50°C.

The sample preparation for studying the total content of amino acids. In a vial (B) add 0.20 ml of the extract. Then pour 3 ml of 6 N aqueous solution of hydrochloric acid containing 0.4% β-mercaptoethanol into the vial. Close the vial hermetically and keep for 24 h at the temperature of 110°C.

Centrifuge and filter the vial with samples. Collect the filtrates into reaction vials: 100 µl from vial A and 20 µl from vial B, place in a vacuum desiccator at a temperature of 40-45ºC and pressure of 1.5 mm Hg and keep to complete removal of hydrochloric acid. Then into the vial for analysis successively add 200 µl of 0.8 M borate buffer with pH 9.0, 200 µl of 20 mM solution of 9-fluorenylmethoxycarbonyl chloride in acetonitrile with an automatic injector, after a 10 min exposure into the reaction vial add 20 µl of 150 mM solution of amantadine hydrochloride in 50% water acetonitrile [7, 8].

Identification of amino acids was performed by retention time of standards. Calculation of the content of bound amino acids was carried out by subtracting the content of free amino acids from their total content.

The preliminary identification of monosaccharides was performed using paper chromatography by the descending method in the system of *n*-butanol – acetic acid – water (4:1:2) with authentic samples of neutral monosaccharides. Chromatograms were developed with the solution of aniline phthalate.

The analysis of sugars was carried out using a chromatograph by Agilent Technologies company (model 1100) equipped with a flow vacuum degasser G1379A, a 4-channel pump of the low pressure gradient G13111A, an automatic injector G1313A, a column thermostat G13116A, refractometric detector G1316A. To carry out the analysis a "Supelcogel-C610H" carbohydrate chromatographic column with the size of 7.8×300 mm was used, and the following chromatographic mode was set: the rate of the mobile phase supply – 0.5 ml/min, eluent – 0.1% aqueous solution of H_3PO_4, working pressure of the eluent – 33-36 kPa, the temperature of the thermostat column – 30°C, the sample volume – 5 µl. Parameters of refractometric detection were as follows: the scale of measurement –1.0, the scan time – 0.5 s. Identification of sugars was carried out according to the retention time of standards.

To analyze bound sugars the acid hydrolysis was carried out according to the following procedure. Into a 5 ml glass vial introduce 400 mg of the extract (accurate weight) add 5 ml of 6 M solution of hydrochloric acid. After that close the vial hermetically and keep for 24 h at the temperature of 100°C in the oven. After cooling centrifuge and filter the vial content through a teflon membrane filter with the pore size of 0.45 µm into a vial for analysis.

Results and Discussion

Using the paper chromatography method, four amino acids have been determined in the extract.

The results of determination of the qualitative composition and quantitative content of free and bound amino acids in the dry extract of *Salvia officinalis* leaves obtained by complex processing using HPLC are presented in Table.

As a result of studying the amino acid composition of the dry extract from *Salvia officinalis* leaves 10 free and 12 bound amino acids have been identified, six of them are indispensable – threonine, valine, isoleucine, leucine, phenylalanine and arginine. As can be seen from Table, in the extract of *Salvia officinalis* leaves dominant substances are tyrosine, serine, glutamic and aspartic acid. The content of free amino acids is 0.38%, and the content of bound amino acids is 0.43%.

Glucose and galactose, and after hydrolysis arabinose, have been identified in the extract.

As a result of determination of the qualitative composition and quantitative content of sugars in the dry extract from *Salvia officinalis* leaves by HPLC four monosaccharides such as glucose, galactose and rhamnose, and after hydrolysis arabinose, have been identified. The content of monosaccharides in the dry extract from *Salvia officinalis* leaves is 6.9%, and after hydrolysis it increases up to 11.2%.

CONCLUSIONS

The amino acid and monosaccharide composition of a dry extract from *Salvia officinalis* leaves obtained by complex processing has been studied; in particular, 10 free and 12 bound amino acids have been identified, six of them are indispensable – threonine, valine, isoleucine, leucine, phenylalanine and arginine, and 4 monosaccharides – glucose, galactose, rhamnose and arabinose.

In the dry extract of *Salvia officinalis* leaves the content of free (0.38%) and bound (0.43%) amino acids, monosaccharides (6.9%) increasing up to 11.2% after hydrolysis has been determined.

REFERENCES

1. *Вовк Г.В., Кошовий О.М., Комісаренко А.М. // Зб. наук. праць співроб. НМАПО ім. П.Л.Шупика. – 2014. – №4. – С. 237-240.*
2. *Державна фармакопея України / Державне підприємство «Науково-експертний фармакопейний центр». – 1-е вид. – Доп. 2. – Х.: ДП «Науково-експертний фармакопейний центр», 2008. – 620 с.*
3. *Компендиум 2014 – лекарственные препараты / Под ред. В.Н.Коваленко. – К.: МОРИОН, 2014. – 2700 с.*
4. *Кошевой О.Н., Вовк Г.В., Ахмедов Э.Ю. и др. // Азербайджанский фармац. и фармакотерапевт. журн. – 2015. – №1. – С. 30-34.*
5. *Пат. України на корисну модель № 92310, МПК A 61 K 36/537, A 61 P 31/00. Спосіб одержання засобу з листя шавлії лікарської з антимікробною та протизапальною активністю / Г.В.Вовк, О.М.Кошовий, А.М.Ковальова, В.А.Рибак, А.М.Комісаренко, М.М.Мига. – № и 2014 02605. Заявл.: 14.03.2014. Опубл.: 11.08.14. – Бюл. №15. – 4 с.*
6. *European Pharmacopoeia. – 4-th ed. – Strasbourg: Council of Europe, 2001. – 2416 p.*
7. *Jámbor A., Molnár-Perl I. // J. of Chromatography A. – 2009. – Vol. 1216. – P. 3064-3077.*
8. *Jámbor A., Molnár-Perl I. // J. of Chromatography A. – 2009. – Vol. 1216. – P. 6218-6223.*
9. *Velickovic A.L., Smelecerovic A.A., Randjelovic N.V. et al. // J. Serb. Chem. Soc. – 2003. – Vol. 68, №1. – P. 57-63.*
10. *WHO monographs on selected medicinal plants: – Geneva: World Health Organization, 2002. – Vol. 2. – 586 p.*
11. *Yinrong Lu, Foo L. Yeap. // Phytochemistry: The Intern. J. of Plant Biochemistry. – 1999. – Vol. 51, №1. – P. 57-63.*
12. *Yudicheva D.M., Vovk G.V., Koshoviy O.M. // Topical issues of new drugs development. – 2015. – P. 109-110.*

АМІНОКИСЛОТНИЙ ТА МОНОЦУКРОВИЙ СКЛАД СУХОГО ЕКСТРАКТУ З ЛИСТЯ ШАВЛІЇ ЛІКАРСЬКОЇ, ОТРИМАНОГО ШЛЯХОМ КОМПЛЕКСНОЇ ПЕРЕРОБКИ

Г.В.Вовк, О.М.Кошовий, А.М.Комісаренко

***Ключові слова**: шавлія лікарська; листя; сухий екстракт; амінокислота; моноцукор; комплексна переробка*

Досліджено амінокислотний та моноцукровий склад сухого екстракту листя шавлії лікарської, одержаного шляхом комплексної переробки. Попереднє виявлення амінокислот та моноцукрів проводили методом паперової хроматографії. Якісний склад та кількісний вміст вільних та зв'язаних амінокислот та моноцукрів у екстракті з листя шавлії лікарської проводили за допомогою високоефективного рідинного хроматографа фірми Agilent Technologies (модель 1100), укомплектованого проточним вакуумним дегазатором G1379A, 4-и канальним насосом градієнта низького тиску G13111A, автоматичним інжектором G1313A, термостатом колонок G13116A, діодноматричним детектором G1316A. В результаті хроматографічного дослідження амінокислотного та моноцукрового складу сухого екстракту листя шавлії лікарської ідентифіковано 10 вільних та 12 зв'язаних амінокислот, шість з яких є незамінними – треонін, валін, ізолейцин, лейцин, фенілаланін і аргінін, та 4 моноцукри – глюкоза, галактоза, рамноза та арабіноза. В екстракті листя шавлії лікарської домінуючими амінокислотами є тирозин, серин, глутамінова та аспарагінова кислоти. В сухому екстракті листя шавлії лікарської встановлено вміст вільних (0,38%) та зв'язаних амінокислот (0,43%), моноцукрів (6,9%), вміст яких після гідролізу збільшується до 11,2%.

АМИНОКИСЛОТНЫЙ И МОНОСАХАРИДНЫЙ СОСТАВ СУХОГО ЭКСТРАКТА ИЗ ЛИСТЬЕВ ШАЛФЕЯ ЛЕКАРСТВЕННОГО, ПОЛУЧЕННОГО КОМПЛЕКСНОЙ ПЕРЕРАБОТКОЙ

Г.В.Вовк, О.Н.Кошевой, А.Н.Комиссаренко

***Ключевые слова**: шалфей лекарственный; листья; сухой экстракт; аминокислота; моносахариды; комплексная переработка*

Исследован аминокислотный и моносахаридный состав сухого экстракта из листьев шалфея лекарственного, полученного путем комплексной переработки. Предварительное определение аминокислот и моносахаридов проведено методом бумажной хроматографии. Качественный состав и количественное содержание свободных и связанных аминокислот и моносахаридов в экстракте из листьев шалфея лекарственного проводилось с помощью высокоэффективного жидкостного хроматографа фирмы Agilent Technologies (модель 1100), укомплектованного проточным вакуумным дегазатором G1379A, 4-х канальным насосом градиента низкого давления G13111A, автоматическим инжектором G1313A, термостатом колонок G13116A, диодноматричным детектором G1316A. В результате хроматографического исследования аминокислотного и моносахаридного состава сухого экстракта листьев шалфея лекарственного идентифицировано 10 свободных и 12 связанных аминокислот, шесть из которых являются незаменимыми – треонин, валин, изолейцин, лейцин, фенилаланин и аргинин, и 4 моносахарида – глюкоза, галактоза, рамноза и арабиноза. В экстракте листьев шалфея лекарственного доминирующими веществами являются тирозин, серин, глутаминовая и аспарагиновая кислоты. В сухом экстракте листьев шалфея лекарственного установлено содержание свободных (0,38%) и связанных аминокислот (0,43%), моносахаридов (6,9%), содержание которых после гидролиза увеличивается до 11,2%.

ТЕХНОЛОГІЯ ЛІКАРСЬКИХ ПРЕПАРАТІВ

Recommended by Doctor of Pharmacy, professor O.M.Kotenko

UDC 615.1:615.456+615.7

THE SUBSTANTIATION FOR INTRODUCTION OF THE INNOVATIVE TECHNOLOGICAL PROCESS FOR INDUSTRIAL PRODUCTION OF PARENTERAL DOSAGE FORMS BY THE EXAMPLE OF TORASEMIDE, SOLUTION FOR INJECTION

A.M.Goy

JSC «Farmak», Kyiv

Key words: parenteral dosage forms; Torasemide; solution for injection; innovative technological process; algorithm; monitoring; critical parameters of the technological process; risk management of pharmaceutical production

The introduction of the innovative technological process for industrial production of parenteral dosage forms by the example of Torasemide, solution for injection has been substantiated in the article. The experimental studies for improving the manufacturing technology of Torasemide, solution for injection, 5 mg/ml, the choice of the filter material, the scientific substantiation of the innovative technological process are presented. In particular, the aseptic conditions of production have been selected using sterile filtration and thermal sterilization (stabilization), the algorithm of monitoring of the critical parameters has been developed using the elements of risk management design of pharmaceutical production.

Creation of new effective parenteral dosage forms is a topical research and practical issue of pharmaceutical industry. In the context of this problem the innovative changes of pharmaceutical industrial production of sterile and aseptic dosage forms for injection require special attention. New active pharmaceutical ingredients, which differ in solubility and absorption affecting substantially bioavailability and therapeutic efficiency of a medicinal form, are not always stable in the process of thermal sterilization and further storage after sterilization. This leads to the necessity to improve the technology and introduce innovative approaches to sterile and aseptic production of parenteral dosage forms.

The history of studying and practical application of synthetic diuretics goes back more than 50 years, the last decades are marked with appearance of diuretics with new pharmacological properties [5].

Nowadays diuretics gain importance in treating congestive heart failure (CHF) and arterial hypertension in many countries, as well as in diseases of the liver and kidneys accompanied with edema. Diuretics are medications, which are more similar to those ones that can be suitable for the prolonged and continuous treatment of arterial hypertension. Diuretic drugs are used for reduction of edemas caused by water and salt retention in the body occurred in diseases of the heart, liver, kidneys and lungs. Mild diuretics are used in combination with other drugs in treating hypertension. Most diuretics often cause the electrolyte imbalance, washout of potassium salts from the body; therefore, simultaneously a patient is prescribed drugs, which contain potassium salts, in order to renew their deficiency in the body. Loop diuretics are the most potent; they block activity of special Na+/2Cl-/K+ transporter in the tubular cells along the whole ascending segment of the Henle's loop. The diuretic action of these drugs is the strongest (the increase of diuresis in an adequate dose exceeds 100% of the original value) and remains even in the reduced renal function (the creatinine clearance is not below 5 ml/min) [1, 5].

Secondary properties of loop diuretics are the ability to reduce moderately the carbonic anhydrase activity and stimulate the synthesis of renal vasodilator prostaglandins, first of all, prostaglandin E2 (PGE2). The increase of synthesis of PGE2 is accompanied by the additional increase of sodium reabsorption in the ascending part of the Henle's loop and decrease of free water reabsorption in collecting tubules. Thanks to such complex of effects loop diuretics are considered to be essential in the treatment of severe CHF.

The regular use of active diuretics provokes electrolyte disorders (the loss of potassium and magnesium), development of metabolic alkalosis. Besides, the constant use of loop drugs reduces their effect. It predetermines replacement of drugs of the same group [5].

Torasemide is the most effective modern loop diuretic. Bioavailability of Torasemide is twice as high as reference drug Furosemide (reaches 80-90% even in CHF). In comparative studies with Furosemide in 234 patients

Torasemide lowered the risk of hospitalizations by 52% related to CHF exacerbation. Moreover, when examining 2303 patients with CHF who received Torasemide or Furosemide randomized in the ratio of 1:1 the reliable decrease of cardiovascular (53%, $p<0.013$) and general (41%, $p=0.035$) mortality was shown [1, 2].

The aim is to substantiate introduction of the innovative technological process for industrial production of parenteral dosage forms by the example of Torasemide, solution for injection.

Materials and Methods

Innovative implementations are difficult and expensive since development of an innovative molecule, a medicine, a dosage form has a number of difficulties. Some of them are: the targeted action focused on the physiological process, organ, system; the slower or increased action as a result of bioavailability; solubility and absorption; insufficient efficiency of the dosage form selected; a negative reactivity of the body, side effects; a degree of danger in use by patients with concomitant pathologies; in pediatric practice and gerontology; instability of parenteral solutions.

Difficulties related to industrial production are: economic costs for modernization of technological equipment and manufacturing processes, transfer of pharmaceutical technologies, and high competition.

The active pharmaceutical ingredient (AFI) – Torasemide is described in leading Pharmacopoeias (EP 6.0, USP 31, and British Ph. 2007). The scientific substantiation of the optimal composition of Torasemide, solution for injection, in the conditions of the technological laboratory of pharmaceutical production was performed using Torasemide substance produced by "Hubei Biocause Pharm. Co. Ltd." firm, China, and it fully complies with the requirements of EP 6.0.

Torasemide is a white or almost white powder, practically insoluble in water, slightly soluble in 96% alcohol, sparingly soluble in diluted solutions of alkali metal hydroxides and slightly soluble in diluted solutions of acids. From patented sources it is known that Torasemide exists in two forms: crystalline and amorphous. Torasemide shows polymorphism: there are four crystalline forms with different physical and chemical properties, in particular the melting point. To develop a medicine – Torasemide, solution for injection, 5 mg/ml, a crystalline substance was used.

Thus, for improvement of the technological process it was necessary to transform of Torasemide API from the insoluble form into the soluble one. In turn, it led to introduction of such excipients into the dosage form as organic solubilizers, buffer system components for stabilization of the solution, pH adjustor, solvents to form the solution for injection (Tab. 1). In our study it has been found that the most stable solutions of Torasemide for injection are obtained by introducing, in addition to the organic solvent, physiologically compatible alkaline buffer solutions of sodium, potassium or ammonia salts, weak acids, such as carbonates, phosphates, glycinates or arginase, N-methylglucosamine or other amino acids. The qualitative composition of excipients required for improvement of the manufacturing technology of Torasemide, solution for injection, is given in Tab. 1.

Table 1

The qualitative composition of excipients required for improvement of the manufacturing technology of Torasemide, solution for injection

Excipients	Functional purpose
Polyethyleneglycol 400	Organic solvent for preparation of injectable dosage forms
Trometamol	Component of the buffer system, stabilizer, pH adjustor
Sodium hydroxide	Component of the buffer system, stabilizer, pH adjustor
0.2 M sodium hydroxide solution	Component of the buffer system, stabilizer, pH adjustor
Water for injection	Solvent for preparation of injectable forms

Table 2

Physical and chemical properties of Torasemide, solution for injection, 5 mg/ml, depending on the filter material

Filter brand	pH of the solution	Content of Torasemide, mg/ml
Before filtration	9.34	4.95
Polyethersulfone "Supor", Pall	9.30	4.92
Polyvinylidentetrafluoride "VDF", Pall	9.32	4.93
Neylon-66 "N-66", Pall	9.12	4.65

The substance of Torasemide for the model batch of Torasemide, solution for injection, 5 mg/ml, is micronized with the micronization level of particles – 90% less than 5 μm. The drug substance and excipients are fully compatible with each other and do not form complexons that can adversely affect the efficacy of the drug.

To determine the mutual influence of the drug and filter materials that can cause a change of physical and chemical properties of the solution the filter materials widely used in production of solutions for injection were studied. The type of the filter material has also a considerable effect on indicators of "Sterility" and "Absence of Particulate Matters" tests. For this purpose, based on the optimal composition, the drug solution was prepared, filtered through various brands of membrane filters with particle size of 0.22 μm and analyzed according to the basic physical and chemical parameters. The research results are presented in Tab. 2.

According to the research data the filter material based on polyvinylidentetrafluoride and polyethersulfone was chosen for development of the technological process.

The selection of the thermal sterilization method was conducted in accordance with the requirements for sterilization of medicinal products developed by the Committee for Medicinal Products for Human Use in the Guideline CPMP/QWP/054/98 "Decision of trees for the selection of sterilization methods".

Table 3

Quality indicators of the model batch of Torasemide, solution for injection, 5 mg/ml, and the reference drug Trifas 20

Model batch /Reference drug	Before sterilization					After thermal sterilization (121°C, 15 min)				
	Content, mg/ml	Impurities				Content, mg/ml	Impurities			
		A: not more than 0.1%	B: not more than 1.2%	C, %	D, %		A: not more than 0.1%	B: not more than 1.2%	C, %	D, %
MC111207 Torasemide, solution for injection, 5 mg/ml, (JSC "Farmak")	5.02	–	0.05	–	–	4.70	0.10	4.67	–	–
61001 "Trifas 20" (A. MENARINI MANUFACT. Italy)	4.80	–	0.51	–	–	4.44	0.07	5.28	–	–
63004 "Trifas 20" (A. MENARINI MANUFACT. Italy)	4.78	–	0.70	–	–	4.48	0.06	5.57	–	–
71005 "Trifas 20" (A. MENARINI MANUFACT. Italy)	4.84	–	0.64	–	–	4.52	0.05	5.37	–	–

Thus, to clarify the peculiarities of sterilization the model batch MC111207 Torasemide, solution for injection, 5 mg/ml, in ampoules was sterilized at 121°C for 15 min (the pharmacopeial mode of thermal sterilization), and the changes of the main quality indicators (the quantitative content of Torasemide, impurities – decomposition products of Torasemide) were observed [2, 3, 4].

For comparison ampoules of the reference drug Trifas 20, solution for injection, manufactured by "A. MENARINI MANUFACTURING" company, Italy were also sterilized at 121ºC for 15 min. The results of the experiment are presented in Tab. 3.

On the basis of the experimental results it can be concluded that improvement of the technological process by transformation of Torasemide in a soluble form, introduction of the solubilizer in the formulation and the buffer mixture for solution stabilization positively affect the drug stability, but the pharmacopeial mode of thermal sterilization causes the increase of impurities and exceeds their permissible content.

Thus, the necessity of introduction of innovative changes in the technological process was stated, namely the optimal method of thermal sterilization according to the requirements for sterilization of medicinal products in the Guideline CPMP/QWP/054/98 "Decision of trees for the selection of sterilization methods".

The next step of the drug development was the process of selection of the temperature conditions for thermal sterilization of the drug in ampoules. For this purpose the solution in ampoules was sterilized at the different modes of sterilization and, based on the results of the quantitative content of Torasemide and impurities in the drug (the drug model batch – MC111207 Torasemide, solution for injection, 5 mg/ml, in ampoules) after sterilization the optimal mode of thermal sterilization was chosen. The results of the experiment are presented in Tab. 4.

Based on the results of the experimental study of the temperature conditions of thermal sterilization for model series batch MC111207 Torasemide, solution for injection, 5 mg/ml, in ampoules, the optimal mode of thermal stabilization of aseptic drug manufacturing – sterile filtration and thermal sterilization (stabilization) has been determined; it fully provides the drug quality by the indicator for "Sterility" test.

Taking into account the overall results of the abovementioned experiments the innovative changes of the technological process for manufacturing Torasemide, solution for injection, 5 mg/ml, have been scientifically substantiated. The aseptic conditions of production have been selected using sterile filtration and thermal sterilization (stabilization) at 105ºC for 15 min. The algorithm of monitoring of the critical parameters of the technological process for manufacturing Torasemide, solution for injection, 5 mg/ml, has been developed.

The algorithm of monitoring of the critical parameters of the technological process for manufacturing Torasemide, solution for injection, 5 mg/ml:

When manufacturing Torasemide solution it is necessary to conduct bubbling of the solution with nitrogen within the whole period of loading and dissolution of Torasemide and before each sampling.

1. Critical parameter – the substance of Torasemide is easily oxidized, and it affects stability of the product; therefore, the whole technological process is conducted under the inert gas flow.

Water for injection is loaded with the temperature of 25ºC into the reactor prepared. Polyethyleneglycol 400 and sodium hydroxide are added. They are mixed until the components become completely dissolved.

2. Critical parameter – duration of mixing is sufficient for dissolution of all components of the drug.

Table 4

The results of the experimental study of temperature conditions of thermal sterilization in the model batch MC111207 Torasemide, solution for injection, 5 mg/ml, in ampoules

No. of the drug batch	The quantitative content of Torasemide, mg/ml	Impurities			
		A: no more than 0.1%	B: no more than 1.2%	C, %	D, %
Model batch MC111207 Torasemide, solution for injection, 5 mg/ml	After thermal sterilization (110°C, 20 min)				
	4.78	0.08	1.9	–	–
	After thermal sterilization (105°C, 30 min)				
	5.01	–	1.3	–	–
	After thermal sterilization (105°C, 15 min)				
	5.01	–	0.77	–	–

Torasemide is loaded and suspended by mixing for 30 min at increased speed of the mixer.

3. Critical parameter – the moisture content in the substance (the loss on drying, %) – not more than 0.5%.

4. Critical parameter – duration of mixing required for obtaining a homogeneous solution.

Stabilization of the solution (pH 9.25-9.4).

5. Critical parameter – pH of the solution.

6. Critical parameter – duration of mixing.

In-process control of the intermediate product. In case of positive testing results the solution is filtered in a sterile tank.

7. Critical parameter – the filter membrane material that can cause the change of physical and chemical properties of the solution.

8. Critical parameter – leaktightness of filters, test for leaktightness before and after the filtration stage.

The solution filtered is poured into 2 ml or 5 ml ampoules made of transparent glass.

9. *Critical parameter* – the volume of the unit (ampoules) content checked during the manufacturing process.

10. *Critical parameter* – providing of hermetic sealing of ampoules.

11. *Critical parameter* – control of the drug solution for the absence of particulate matters.

Ampoules with the drug solution are sterilized (stabilized).

12. *Critical parameter* – temperature and duration of sterilization affecting physical and chemical properties of the drug.

CONCLUSIONS

1. In the process of the experimental research it has been found that the technological process must be improved by transformation of Torasemide API from the insoluble form into the soluble one. The selection and introduction of excipients have been carried out. They are: a solubilizer – Polyethyleneglycol 400; components of the buffer system, stabilizers, pH adjustors – Trometamol and 0.2 M solution of sodium hydroxide, which positively affect the drug stability, are fully compatible with each other and do not form complexons that can adversely affect the efficacy of the drug.

2. According to the research results the filter material based on polyvinylidentetrafluoride and polyethersulfone has been chosen.

3. The innovative technological process for manufacturing Torasemide, solution for injection, 5 mg/ml, has been scientifically substantiated. In particular, the aseptic conditions of production have been selected using sterile filtration, the algorithm of monitoring of the critical parameters of the technological process for manufacturing Torasemide, solution for injection, 5 mg/ml, has been developed.

REFERENCES

1. *Achhammer J., Metz P. // Drugs. – 1991. – Vol. 41. – P. 80-91.*
2. *Engelhardt S., Meineke I., Brockmöller J. // J. of Chromatography B. – 2006. – Vol. 831, Issues 1-2. – P. 31-35.*
3. *European Pharmacopeia 7.5. General Notices apply to all monographs and other texts. – P. 4701-4702.*
4. *Jovic Z., Zivanovic L., Protic A. et al. // J. of Liquid Chromatography and Related Technol. – 2013. – Vol. 36, Issue 15. – P. 2082-2094.*
5. *Masereel B. // Eur. J. of Med. Chem. – 1995. – Vol. 30, №4. – P. 343-351.*

ОБГРУНТУВАННЯ ВПРОВАДЖЕННЯ ІННОВАЦІЙНОГО ТЕХНОЛОГІЧНОГО ПРОЦЕСУ ПРОМИСЛОВОГО ВИРОБНИЦТВА ПАРЕНТЕРАЛЬНИХ ЛІКАРСЬКИХ ФОРМ НА ПРИКЛАДІ ПРЕПАРАТУ ТОРАСЕМІД, РОЗЧИН ДЛЯ ІН'ЄКЦІЙ

А.М.Гой

Ключові слова: *парентеральні лікарські форми; Торасемід; розчин для ін'єкцій; інноваційний технологічний процес; алгоритм; моніторинг; критичні параметри технологічного процесу; управління ризиками фармацевтичного виробництва*

Висвітлено обгрунтування впровадження інноваційного технологічного процесу промислового виробництва парентеральних лікарських форм на прикладі препарату Торасемід, розчин для ін'єкцій. Представлені експериментальні дослідження з удосконалення технології препарату Торасемід 5 мг/мл розчин для ін'єкцій, з вибору фільтрувального матеріалу, наукового обґрунтування інноваційного технологічного процесу, а саме: обрані асептичні умови виробництва із застосуванням стерильної фільтрації і термічної стерилізації (стабілізації), алгоритм моніторингу критичних параметрів з використанням елементів проектування управління ризиками фармацевтичного виробництва.

ОБОСНОВАНИЕ ВНЕДРЕНИЯ ИННОВАЦИОННОГО ТЕХНОЛОГИЧЕСКОГО ПРОЦЕССА ПРОМЫШЛЕННОГО ПРОИЗВОДСТВА ПАРЕНТЕРАЛЬНЫХ ЛЕКАРСТВЕННЫХ ФОРМ НА ПРИМЕРЕ ПРЕПАРАТА ТОРАСЕМИД, РАСТВОР ДЛЯ ИНЪЕКЦИЙ

А.М.Гой

Ключевые слова: *парентеральные лекарственные формы; Торасемид; раствор для инъекций; инновационный технологический процесс; алгоритм; мониторинг; критические параметры технологического процесса; управление рисками фармацевтического производства*

Обосновано внедрение инновационного технологического процесса промышленного производства парентеральных лекарственных форм на примере препарата Торасемид, раствор для инъекций. Представлены экспериментальные исследования усовершенствования технологии получения препарата Торасемид 5 мг/мл раствор для инъекций, технологии выбора фильтровального материала, научного обоснования инновационного технологического процесса, а именно: выбраны асептические условия производства с применением стерильной фильтрации и термической стерилизации (стабилизации), алгоритм мониторинга критических параметров с использованием элементов проектирования управления рисками фармацевтического производства.

Recommended by Doctor of Pharmacy, professor I.I.Baranova

UDC 615.451.124.014.23

THE STUDY OF PROPERTIES OF POLYMERIC STABILIZED EMULSIONS BASED ON ARISTOFLEX

T.Kovaliova, N.Polovko

National University of Pharmacy

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

The present work contains the study of stabilizing properties of Aristoflex AVC polymeric viscosity modifier and stabilizer of the oil/water type emulsion systems. The technological, physicochemical and rheological studies of emulsions on basis of the Aristoflex polymeric viscosity controller have been conducted. It has been proven that they are structured disperse systems with certain thixotropic properties. The results of the research indicate the prospects of using Aristoflex as a monostabilizer in the concentration of 1–2.5% with the oil phase content of 5–30%, respectively. It has been found that introduction of Aristoflex to the water or oil phase does not affect their organoleptic, physicochemical and rheological properties.

An important issue in the industrial production of therapeutic and cosmetic creams is their stability during storage; therefore, the rational selection of stabilizing components is of current importance at the stage of development and planning of the cream production. The traditional practice of obtaining emulsion systems is based on the use of surfactants, which role consists in decreasing the interphase tension, as well as in combining surfactants with gelling agents that raise the stability of emulsions thanks to the ability to form viscous high-tensile colloidal structures. However, there are data on the irritating effect of creams on the basis of conventional emulsifiers on the skin, and it makes the search and study of new stabilizing additives topical [2, 4, 5].

In recent years a number of publications described the experience of application of stabilizers combining electrostatic and steric mechanisms of emulsion stabilization [6]. Such substances have a polymeric nature and can stabilize emulsion systems as monoemulsifiers. Having the properties of anionic surfactants such polymers prevent the loss of the aggregate resistance of emulsions due to electrostatic repulsion of disperse phase particles, and their branched spatial structure provides the steric stabilization of emulsions with formation of a gelatinous structure in the zone of overlapping adsorption and solvation layers [3, 6-8].

The aim of this work was to study organoleptic, structural and mechanical properties of emulsion bases stabilized by a copolymer of acrylamido-methyl-propane-sulfonic acid and vinyl-pyrrolidone (Aristoflex AVC, ClariantSurfactants, Germany), hereinafter referred to as Aristoflex. Aristoflex belongs to the group of modern viscosity modifiers and stabilizers of emulsion systems of the oil/water type where it is used both in combination with conventional emulsifiers and as a monostabilizer makingdermatologically soft cream-gel systems known as surfactant-free without the irritating effect on the skin [9-11].

Materials and Methods

The subjects of the study were two batches of emulsion bases of the oil/water type prepared by the cold emulsification method with variations of the content of oil with the constant quantity of the emulsifier in the first batch and the content of the emulsifier with the constant quantity of the oil phase in the second batch.

The colloidal and thermal stability were determined in accordance with the methods of the State Standard (GOST) for "cosmetic creams". The type of emulsion was determined by the dilution method. The pH indicators of the experimental samples were determined by potentiometry in 10% water extraction of the cream by a MI pH meter (Russia) with pH 150 according to the II ed. of the State Pharmacopoeia of Ukraine (SPhU) [1]. The rheological studies were conducted with a BROOKFIELD HB DV-II PRO viscosimeter (USA) within the range of the shear rate from 18.6 sec^{-1} to 93 sec^{-1} (SC4-21 spindle for 8.3 ml chamber) at the temperature of 20ºC. Based on the measurement results the rheograms of the shear stress (τ) versus the shear rate gradient (Dr), as well as the diagram of the structural viscosity (η) – shear rate (Dr) relationship were built. The presence of thixotropic properties of the samples was determined by appearance of the fluidity curve.

The microscopic analysis was conducted by a "Konus-Akademy" laboratory microscope with a ScopeTek DCM510 ocular camera. The ScopePhoto™ software was used for image visualization.

Results and Discussion

Both technologies proposed by the manufacturer were used while preparing the experimental samples: the preliminary introduction of Aristoflex to the oil phase before its mixing with the water phase and introduction to the water phase. The samples with the same organoleptic, technological and tactile indices were obtained, and subjected to the thermal and colloidal stability tests; as a result, stable samples were selected for further studies (Table).

Table

Experimental samples with Aristoflex AVC and their indices

Sample No.	Aristoflex, %	Oil phase, %	Sensory characteristics of the sample	Structural viscosity mPa·s^{-1}
Batch 1				
1	2.0	5.0	A matte cream of a white colour and medium consistency is absorbed without leaving a trace and stickiness	10200
2	2.0	10.0		12000
3	2.0	15.0	A glossy cream of a white colour and thick consistency is absorbed without leaving a trace and stickiness	12500
4	2.0	20.0		13300
5	2.0	25.0	A glossy cream of a white colour and thick consistency gives a greasy feeling to the skin when applied, is absorbed without leaving a trace and stickiness	15600
6	2.0	30.0		17400
Batch 2				
8	1.0	20.0	A translucent cream-gel is quickly absorbed without leaving a trace and stickiness	4900
10	1.5	20.0	A matte cream of a white colour and thick consistency is absorbed without leaving a trace and stickiness	9500
4	2.0	20.0	A glossy cream of a white colour and thick consistency is absorbed without leaving a trace and stickiness	13300
11	2.5	20.0		15500

It has been found that being within the range of concentrations of 1-2.5% Aristoflex is capable of stabilizing emulsion systems without introduction of an additional emulsifier. The attempts to obtain stable emulsions with 0.5% concentration of Aristoflex and 5% oil concentration resulted in their destruction within the first day after preparation. The samples with the Aristoflex concentration of more than 2.5% were too viscous indicating their low consumer properties.

It is known that the structural viscosity of disperse systems formed with the help of polymeric stabilizers determines their thickening properties. That is why the next step of our investigation was to study the relationship between the structural viscosity of the samples obtained and the Aristoflex concentration (Fig. 1).

As it is shown by the results of the studies, viscosity of the emulsion system under study considerably increased with the increase of the Aristoflex concentration from 1.0% to 2.5%. Therefore, the excipient used as a monostabilizer of the oil/water type emulsions is effective.

The rheograms of the emulsion samples were also built, their hysteresis loop area indicated the thixotropic properties with a different degree of manifestation (Fig. 2, 3).

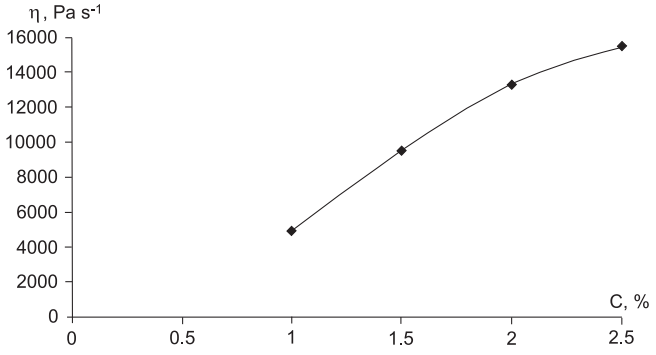


Fig. 1. The relationship of the structural viscosity of the samples and the Aristoflex concentration.

The rheograms built show the uniformity of the flow of the samples under research, as well as their compliance with the non-Newtonian type with plastic properties.

The study of the relationship of the structural viscosity and the shear rate gradient of the emulsion samples with Aristoflex in the concentration of 1-2.5% showed a gradual decrease of the structural viscosity with increase in the shear rate gradient (Fig. 4, 5).

The most intensive decrease of the structural viscosity is observed within the shear rate range from 20 s^{-1} to 40 s^{-1}, then viscosity decrease occurs insignificantly and almost does not change at the deformation rate of 55 s^{-1}. It indicates the structure destruction.

The dispersion degree of particles is one of the criteria determining the consistency and stability of emulsions. It is considered that an optimal particle size of the

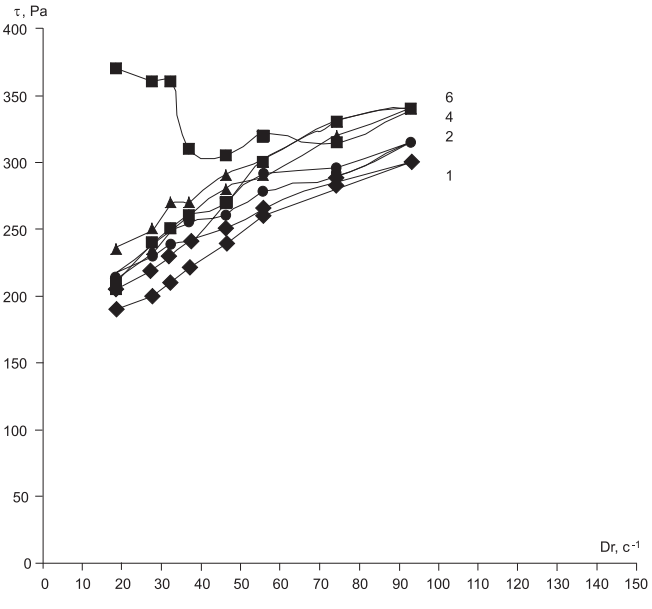


Fig. 2. The rheograms of emulsion samples No. 1, 2, 4, 6 with Aristoflex in 2% concentration with 5–30% concentration of the oil phase.

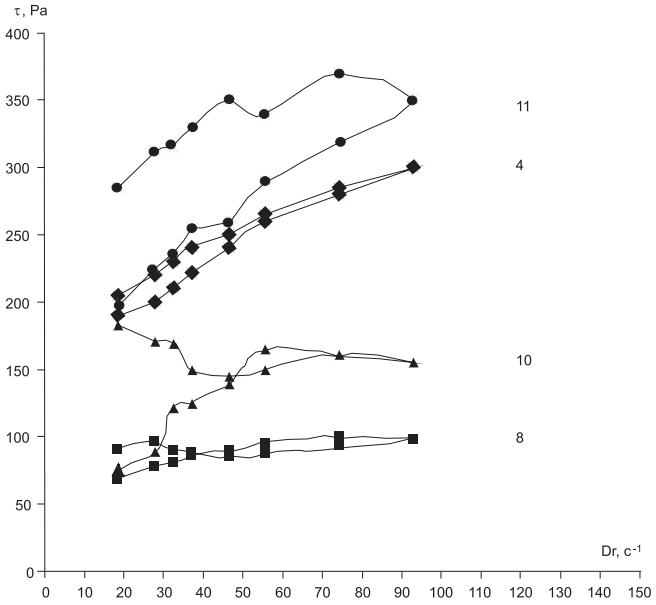


Fig. 3. The rheograms of emulsion samples No. 1, 2, 4, 6 with Aristoflex in 1–2.5% concentration with 20% concentration of the oil phase.

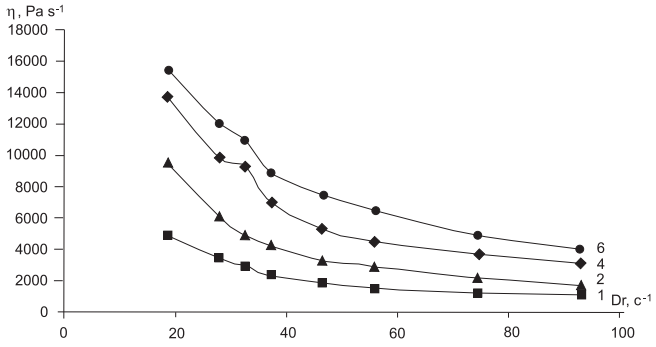


Fig. 4. The relationship of the structural viscosity and the shear rate in the experimental samples with Aristoflex in 2% concentration with 10-30% concentration of the oil phase.

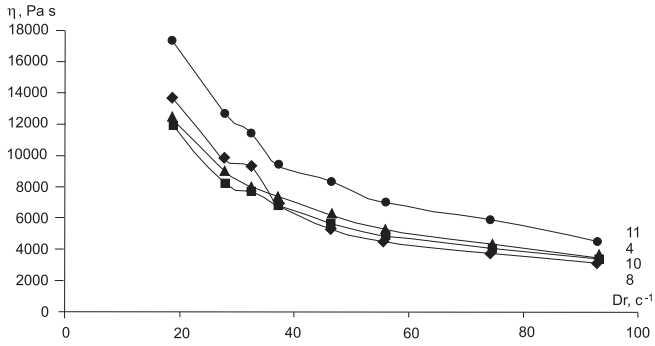


Fig. 5. The relationship of the structural viscosity and the shear rate in the experimental samples with Aristoflex in 1-2.5% concentration with 20% concentration of the oil phase.

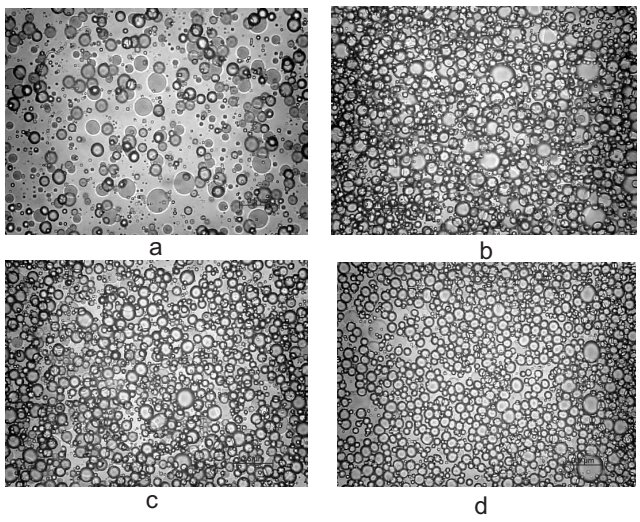


Fig. 6. Dispersity of the particles of the oil phase of the experimental samples where: a – sample 1, b – sample 2, c – sample 6, d – sample 4.

emulsion cream is 1-2 μm. Thus, our next step was the microscopic examination of the experimental emulsion samples; it allowed to determine finally an optimal emulsifier concentration in stable samples (Fig. 6a, b, c, d).

Dispersion analysis of the emulsions with different Aristoflex concentrations has shown that samples No. 1, 2, 6 have a heterogeneous drop size from 1 to 2 μm; in sample No. 1 there is enlargement of the emulsion particles indicating coalescence. Monodispersity and high density of drops in sample No. 4 are the evidence of a concentrated nature of the dispersed phase and allow to refer it to the ultramicroheterogeneous system.

CONCLUSIONS

The technological, physicochemical and rheological studies of emulsions on basis of the Aristoflex polymeric viscosity controller have been conducted. It has been proven that they are structured disperse systems with certain thixotropic properties.

The results of the research indicate the prospects of using Aristoflex as a monostabilizer in the concentration of 1-2.5% with the oil phase content of 5-30%, respectively, the optimal concentration is 2%.

It has been found that introduction of Aristoflex to the water or oil phase does not affect their organoleptic, physicochemical and rheological properties.

REFERENCES

1. *Державна фармакопея України / Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів». – 2-е вид. – Х.: ДП «Український науковий фармакопейний центр якості лікарських засобів», 2014. – Т. 1. – С. 51.*
2. *Дмитрієвський Д.І., Рибачук В.Д., Хоменко В.М. та ін. Допоміжні речовини в технології ліків: вплив на технологічні, споживчі, економічні характеристики і терапевтичну ефективність: Навч. посіб. для студ. вищ. фармац. навч. закл. / За ред. І.М.Перцева. – Х.: Золоті сторінки, 2010. – 600 с.*
3. *Ковальова Т.М. // Вісник фармації. – 2015. – №1 (81). – С. 38-41.*
4. *Кутц Г. Косметические кремы и эмульсии. Состав, методы получения и испытаний. – М.: Косметика и медицина, 2004. – 272 с.*

5. *Структура и текстура пищевых продуктов. Продукты эмульсионной природы / Под ред. М.Б.МакКенна. – С.Пб.: Профессия, 2008. – 471 с.*
6. *Jingyu Shi. Steric Stabilization. – Columbus: The Ohio State University, 2002. – 43 p.*
7. *Loffer M., Miller D., Henning T. [Електронний ресурс] // Household &Personal Products Industry, 2002. – Режим доступу: https://www.highbeam.com*
8. *Manor O., Chau T.T., Stevens G.W. et al. // Langmuir. – 2012. – Vol. 28, №10. – P. 4599-4604.*
9. *Pat. CN102573785 (A), A 61 K8/06, A 61 K8/35. Oil-in-water emulsion with improved sensory properties / Chantal Amalric, Juanshu Shen, J.Guilbot, Rolland H. Gorce A., S.Kerverdo; SEPPIC. – № 20100722; Publ. 2012-07-11.*
10. *Pat. WO2005025522 A2. Skin care composition / Yasuko Nmn Suginaka. – № CA2536204A1. – Appl.: 31.08.2004. Publ.: 24.03.2005.*
11. *Wang L., Fang J. // Central Eur. J. of Energetic Materials. – 2013. – Vol. 10, №1. – P. 87-102.*

ВИВЧЕННЯ ВЛАСТИВОСТЕЙ ПОЛІМЕР-СТАБІЛІЗОВАНИХ ЕМУЛЬСІЙ НА ОСНОВІ ARISTOFLEX

Т.М.Ковальова, Н.П.Половко

***Ключові слова**: емульсія; емульгатор; полімерні регулятори в'язкості; реологічні властивості; структурна в'язкість*

Робота містить дослідження стабілізуючих властивостей полімерного модифікатора в'язкості і стабілізатора емульсійних систем типу олія/вода Aristoflex AVC. Проведені технологічні, фізико-хімічні та реологічні дослідження емульсій на основі полімерного регулятора в'язкості аристофлекс; доведено, що вони є структурованими дисперсними системами з певними тиксотропними властивостями. Результати проведених досліджень свідчать про перспективність використання аристофлекс в якості моностабілізатора в концентрації 1-2,5% при вмісті масляної фази 5-30% відповідно. Виявлено, що введення Aristoflex у водну або масляну фазу не впливає на їх органолептичні, фізико-хімічні та реологічні властивості.

ИЗУЧЕНИЕ СВОЙСТВ ПОЛИМЕР-СТАБИЛИЗИРОВАННЫХ ЭМУЛЬСИЙ НА ОСНОВЕ ARISTOFLEX

Т.Н.Ковалева, Н.П.Половко

***Ключевые слова**: эмульсия; эмульгатор; полимерные регуляторы вязкости; реологические свойства; структурная вязкость*

Работа содержит исследование стабилизирующих свойств полимерного модификатора вязкости и стабилизатора эмульсионных систем типа масло/вода Aristoflex AVC. Проведены технологические, физико-химические и реологические исследования эмульсий на основе полимерного регулятора вязкости аристофлекс; доказано, что они являются структурированными дисперсными системами с определенными тиксотропными свойствами. Результаты проведенных исследований свидетельствуют о перспективности использования аристофлекса в качестве моностабилизатора в концентрации 1-2,5% при содержании масляной фазы 5-30% соответственно. Выявлено, что введение Aristoflex в водную либо масляную фазу не влияет на их органолептические, физико-химические и реологические свойства.

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

UDC 615.014.2 : 615.32 615.244

THE STUDY OF THE EFFECT OF THE CRITICAL PARAMETERS ON THE MANUFACTURING PROCESS OF THE OIL PHYTOEXTRACT WITH THE HEPATOPROTECTIVE ACTION

O.Yu.Tkachuk, L.I.Vyshnevska, T.M.Zubchenko

National University of Pharmacy

Key words: oil extracts; critical parameters; technology; composition of the herbal raw material; hepatoprotector

The experimental studies conducted allow determining the critical parameters and their effect on the manufacturing process of oil extracts of the composition of the herbal raw material with the hepatoprotective action. The influence of the particle size of the raw material, concentration and the amount of ethanol on moistening of the phytocomposition and the temperature of extraction has been studied. As a result of the study the optimal parameters of extraction with the oil extractants providing efficiency of the active substances release from the herbal raw material have been determined. They are grinding of the herbal raw material by a screw shredder, an extractant for moistening is 70% ethanol in the amount of 0.6±0.1 ml per 1.0 of the raw material; the swelling time – 2.0±0.5 h; the temperature of extraction – 55±5ºC. According to the certain critical points of the manufacturing process the flowchart of manufacturing the combined oil herbal medicinal product under the conditional name "Oleosil" has been developed.

Hepatites with the impaired biliary excretion, as well as inflammatory diseases of the liver and gallbladder are the widespread human diseases, first of all among people of the middle and senior age. The timely treatment of the pathologies mentioned prevents development of chronic diseases and improves the quality of the patients' life. Moreover, synthetic and herbal medicines are used [3].

The data obtained in the previous phytochemical studies [7], as well as the data of the systematized literary material [4, 5, 6] have shown the prospects of creating a herbal medicine with the hepatoprotective action on the basis of the phytocomposition (wild carrot seeds, flowers of chamomile and corn silks).

The aim of this research is to study the effect of critical parameters and determine the optimal manufacturing process of oil extracts of the composition of the herbal raw material in order to create the combined oil herbal medicinal product with the hepatoprotective action under the conditional name "Oleosil".

Materials and Methods

The composition of the medicinal herbal raw material containing wild carrot seeds, flowers of chamomile and corn silks in the ratio of (1: 1: 1) was studied. As an extractant the refined corn oil was used [6].

The basic factors affecting the rate and completeness of release of biologically active substances (BAS) were studied. They are the degree of grinding, the type of the extractant and its concentration, the "raw material – extractant" quantitative relationship, the extraction temperature, duration of extraction.

The powdered samples of the herbal raw material were mixed in equal amounts and moistened with ethanol in the concentration from 40 to 96%, stirred to obtain a uniformly moistened mixture of the raw material. To increase the contact surface with the solvent and provide its penetration inside the cell the moistened raw material was placed into a heated infusion cup, covered with a lid and allowed to stand for 2 hours.

Then corn oil heated to 50°C was poured to the moistened raw material to obtain a «mirror» effect and allowed to stand on a water bath for 4 hours. Extraction was conducted at a temperature of 55±5°C. The cooled oil herbal extract was drained off, the raw material was pressed. The extract was clarified by settling for twenty-four hours at a room temperature and further filtration.

The qualitative composition and quantitative content of BAS of the phytocomposition rich in flavonoids, hydroxycinnamic acids, carotenoids, chlorophyll, vitamins, organic and fatty acids was studied by the method of high performance liquid chromatography [7, 10, 12-15].

The studies of factors affecting the yield of BAS were conducted according to the method of quantitative determination of the amount of carotenoids and chlorophylls previously developed [8]. These substances were selected since their content among BAS of the lipophilic nature of the composition of the herbal raw material under research was the greatest [7]. The quantitative content of carotenoids and chlorophylls in oil phytoextracts was determined by the method of UV/VIS absorption spectrophotometry on a "Specord 200" spectrophotometer.

Results and Discussion

During extraction a considerable influence on the speed of the equilibrium achievement in the herbal raw material – solvent system has the degree of the raw material grinding [1, 2]. First of all, the need of grinding is caused by the possibility of improving the extractant penetration

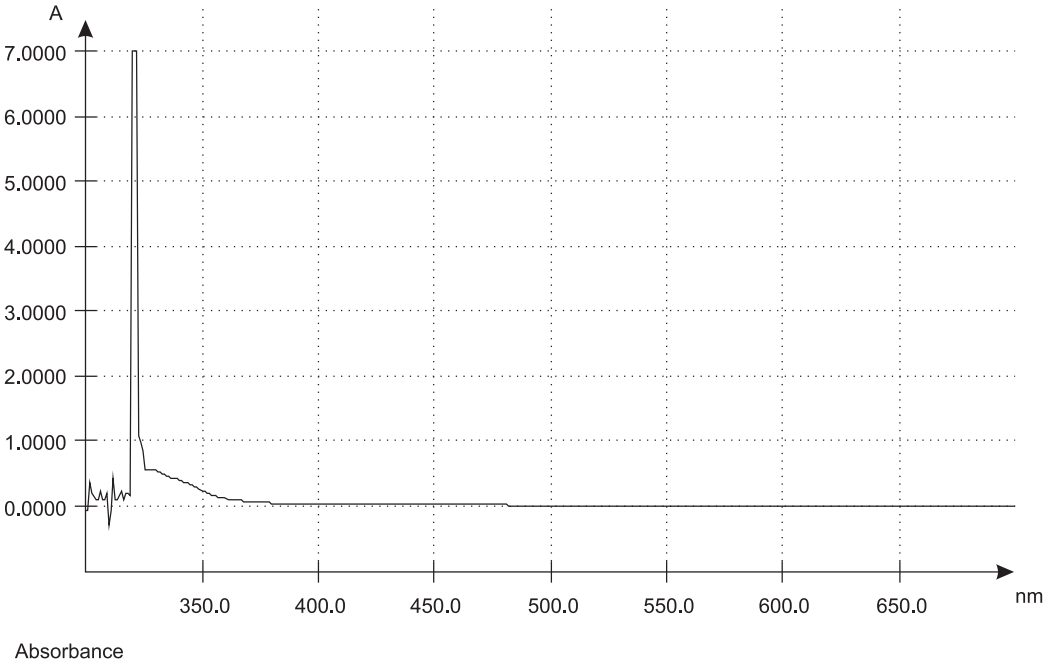


Fig. 1. The absorption spectrum of OE obtained without wetting of the phytocomposition with ethanol.

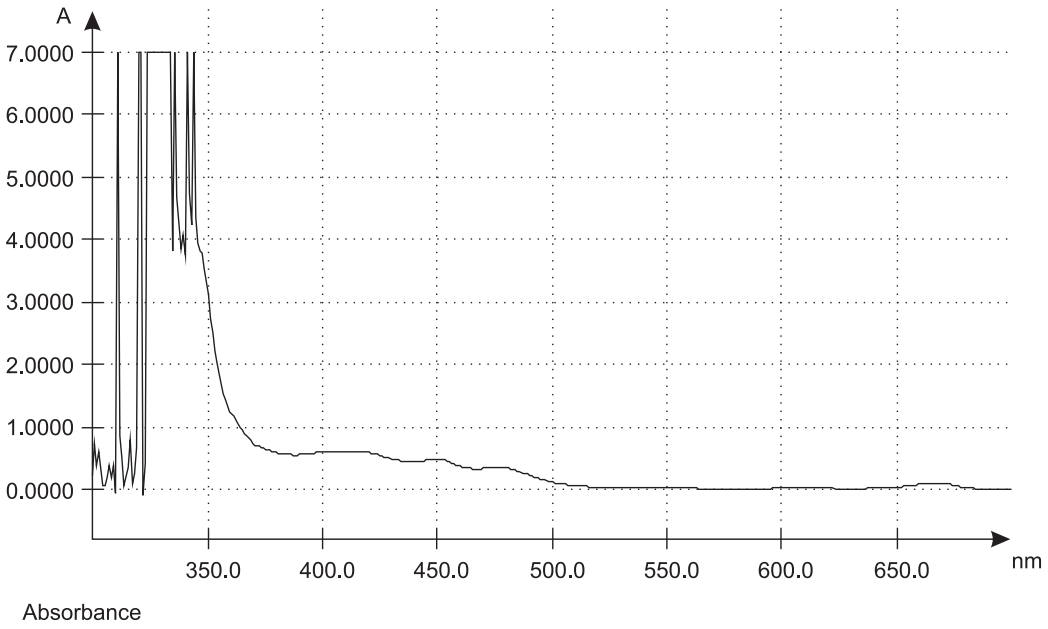


Fig. 2. The absorption spectrum of OE obtained after the preliminary wetting of the phytocomposition with 96% ethanol.

into the layer of the material that has a cellular structure. Cellular coats in seeds are thicker and coarser than in herb, flowers and leaves; therefore, seeds require finer grinding. The best results were achieved while grinding by a screw shredder. Moreover, there was destruction of cells of the raw material with air displacement [1, 2].

It was previously found by us that fat-soluble substances were easily react to form oil extracts, but hydrophilic substances were not practically extracted [4-6]. To transfer BAS with the diphilic nature from the raw material into oil it is reasonable to provide desorption of substances from cells. Intensification of processing of the medicinal herbal raw material is possible when using the system of immiscible solvents [4, 5, 8, 9].

To enrich the oil extract of BAS with the average polarity the preliminary wetting of the composition of the herbal raw material with the water-alcohol solution was proposed. It contributes to weakening of intermolecular bonds, additional hydration of polar groups and hydrophilic compounds. Wetting of the phytocomposition with ethanol improves penetration of the oil solvent inside the cells of the herbal raw material, and it allows to intensify desorption of lipophilic and diphilic BAS. A comparative analysis of absorption spectra of oil extracts (OE) obtained without the preliminary wetting of the raw material and the raw material moistened with ethanol has shown substantial differences in absorption intensity, and it is directly related to the content of biologically active substances (Fig. 1, 2, 3).

It is possible to distinguish three basic absorption maxima in the spectra in Fig. 2 and 3. The first one, at the wavelength of 280-320 nm, can be referred to absorption

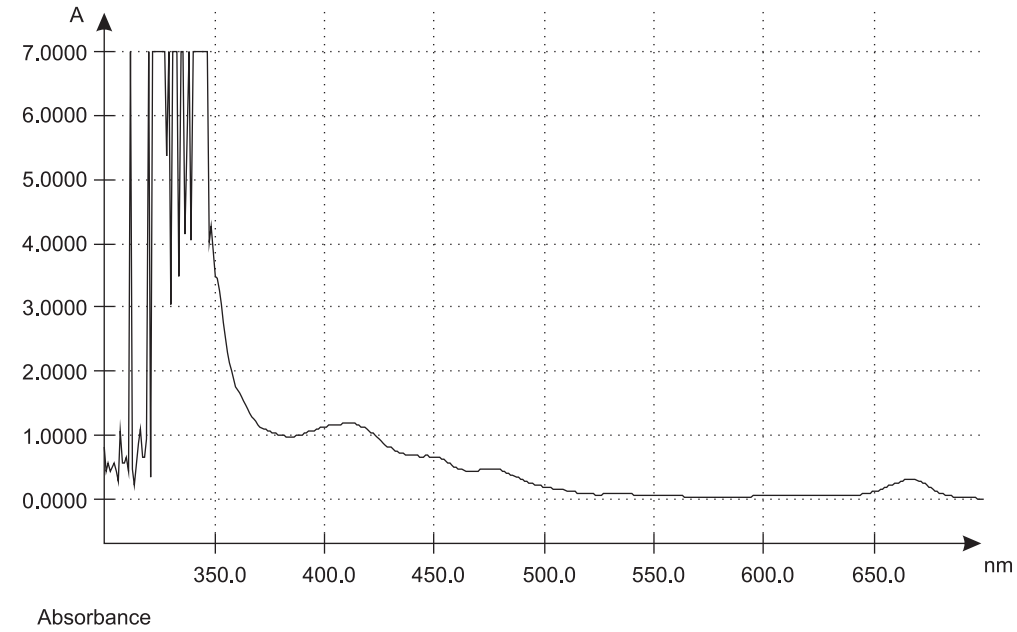


Fig. 3. The absorption spectrum of OE obtained after the preliminary wetting of the phytocomposition with 70% ethanol.

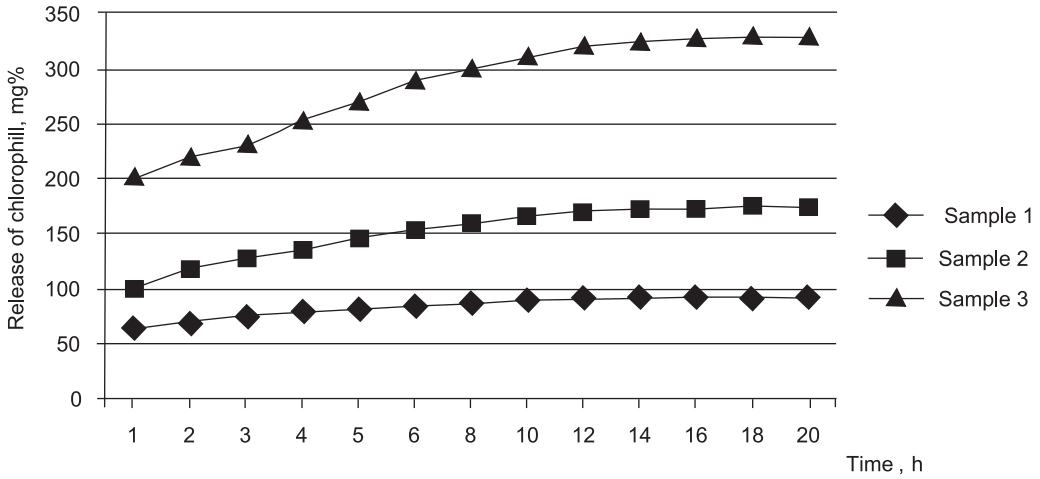


Fig. 4. The content of chlorophyll and its derivatives in oil extracts from the phytocomposition depending on conditions and the time of extraction.

of the hydrophilic complex of BAS of the phytocomposition; the second one, at the wavelength of 450 nm, is typical for carotenoids. The third absorption maximum in the range of 670-685 nm is characteristic for chlorophyll and its derivatives.

Thus, the absorption spectra of the samples of the oil extracts obtained after wetting of the herbal raw material with solutions of ethanol in the concentrations of 40, 70 and 96% evidently demonstrate the possibility of increasing the yield of both diphilic and lipophilic BAS from the phytocomposition containing wild carrot seeds, flowers of chamomile and corn silks. The results of the research indicate that wetting of the phytocomposition with ethanol in the concentration from 40% promotes penetration of the solvent into the raw material cells, its swelling and increase of the desorption degree of not only lipophilic substances (carotenoids, chlorophylls), but polar substances (flavonoids) as well. However, when wetting with 40% ethanol the raw material considerably increases in its volume while swelling and becomes more compact, and it requires more oil for extracting.

Wetting with 96% ethanol promotes better release of lipophilic and hydrophilic compounds (Fig. 2), but the best results were obtained when moistening the phytocomposition with 70% ethanol (Fig. 3).

To determine the dependence of the release of BAS from the phytocomposition on the extraction conditions the lipophilic fraction presented by chlorophyll and its derivatives with the visible absorption maximum at the wavelength of 660-680 nm was chosen.

The quantitative content of lipophilic BAS, in particular chlorophylls and their derivatives, in the samples of the oil extracts obtained depends on the concentration of ethanol used for the preliminary wetting of the phytocomposition (Sample 1 – with 40% ethanol; Sample 2 – with 96% ethanol; Sample 3 – with 70% ethanol), and duration of extraction (Fig. 4). As can be seen from Fig. 3, most BAS react with the extractant for 12-14 hours.

As a result of the research conducted the optimal parameters for extraction with oil extractants providing efficiency of release of active substances from the herbal raw material have been determined. They are grinding

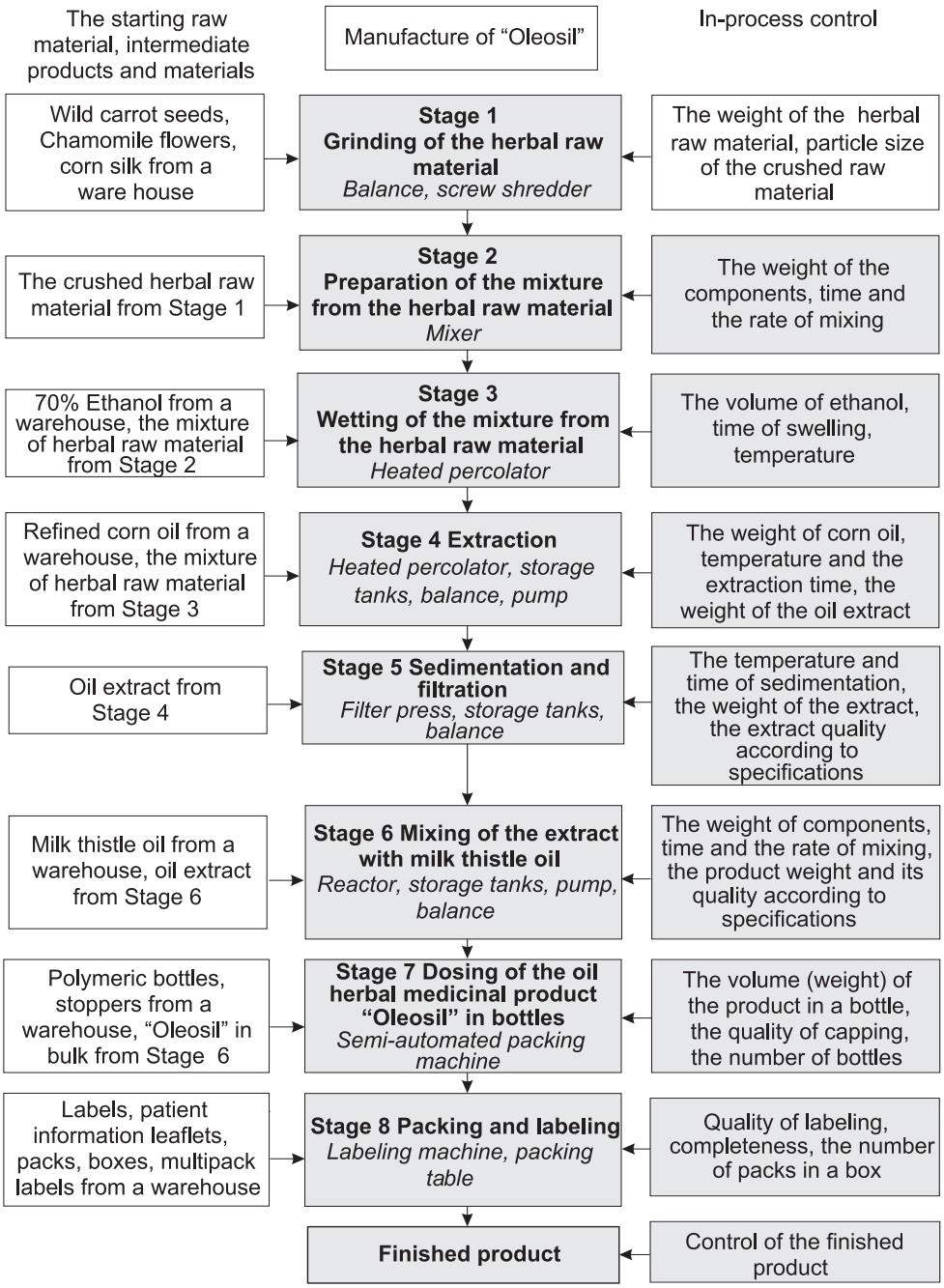


Fig. 5. The flowchart of manufacturing the combined oil herbal medicinal product "Oleosil".

of the herbal raw material by a screw shredder, an extractant for moistening is 70% ethanol in the amount of 0.6±0.1 ml per 1.0 of the raw material; the swelling time – 2.0±0.5 h; the temperature of extraction – 55±5ºC.

In order to increase the therapeutic effect of the product as a medicine with the hepatoprotective action the milk thistle oil obtained from milk thistle (*Silybum marianum*) seeds by direct compression was added to its composition. The milk thistle oil is a classic hepatoprotector – an antioxidant that improves metabolic processes in hepatocytes, increases resistance (stability) of hepatic cells to unfavourable harmful environmental factors.

On the basis of the critical points determined the technology of the complex herbal medicinal product "Oleosil" containing the oil extract from the phytocomposition and milk thistle oil in the ratio of 2:1 and possessing the hepatoprotective action was developed. The flowchart of manufacturing the combined oil herbal medicinal product "Oleosil" is presented in Fig. 5.

CONCLUSIONS

1. The effect of conditions for extraction on release of biologically active substances from the herbal raw material has been studied, and the critical parameters for the technology of the oil herbal extract under the conditional name "Oleosil" have been determined.

2. The optimal conditions for obtaining the oil extract from the phytocomposition that contains wild carrot seeds, chamomile flowers and corn silks in the ratio of (1:1:1) have been substantiated.

3. The flowchart of manufacturing the combined oil herbal medicinal product with the hepatoprotective action under the conditional name "Oleosil" has been developed.

REFERENCES

1. *Дячок В.В., Мальований М.С. // Энерготехнологии и ресурсосбережение. – 2010. – №2. – С. 72-75.*
2. *Дячок В.В., Мальований М.С. // Вопросы химии и хим. технол. – 2009. – №4. – С. 177-180.*
3. *Машковский М.Д. Лекарственные средства: Пособие для врачей в 2-х т. – М.: Новая волна, 2005. – 435 с.*
4. *Наймушина Л.В., Карасева А.Ю., Чесноков Н.В. // J. of Siberian Federal University Chem. – 2012. – №5. – С. 281-288.*
5. *Сорокин В.В., Вайнштейн В.А., Карнаухова И.Е., Чибиляева Т.Х. // Хим.-фармац. журн. – 2008. – Т. 42, №8. – С. 23-25.*
6. *Ткачук О.Ю., Вишневська Л.І., Зубченко Т.М., Бисага Є.І. // Зб. наук. праць співроб. НМАПО ім. П.Л.Шупика. – 2015. – Т. 4, №24. – С. 311-315.*
7. *Ткачук О.Ю., Вишневська Л.І., Зубченко Т.М., Горлачова В.І. // Зб. наук. праць співроб. НМАПО ім. П.Л.Шупика. – 2015. – Т. 5, №2 – С. 234-238.*
8. *Design Qualification Operational Qualification HPLCE quipment Case Study Publication from www.labcompliace com Revision 1.02 May 12.2001.*
9. *Higuers-Ciapara I., Felix-Valenzuella L., Goycoolea F.M. // Grit. Rev. Food Sci. Nutr. – 2006. – Vol. 46. – P. 185-196.*
10. *Huber L. Analytical Instrument Qualification and System Validation // Agilent Technologies Printed in Germany, January 1. 2009. – 61 p.*
11. *Johnson T.J. // Nutr. Clin. Care. – 2002. – Vol. 5. – P. 56-65.*
12. *Rodguez-Amaya D.B., Kimura M. Harvest Techn. Monograph 2. – Washington, DC and Cali: IFPRI and CIAT. Harvest Plus, 2004. – 58 p.*
13. *Solorzano Santos F., Miranda-Noveles M.G. // Current Opinion in Biotechnol. – 2012. – Vol. 23, №2. – P. 136-141.*
14. *Tolouee van T.M., Alinezhad S., Saberi R. et al. // Intern. J. of Food Microbiol. – 2010. – Vol. 139, №3. – P. 127-133.*
15. *Unites States Pharmacopeia, Chapter "1058", Analytical Instrument Qualification, Rockville, USA, 2008.3.*

ВИВЧЕННЯ ВПЛИВУ КРИТИЧНИХ ПАРАМЕТРІВ ВИРОБНИЦТВА НА ТЕХНОЛОГІЧНИЙ ПРОЦЕС ОЛІЙНОГО ФІТОЕКСТРАКТУ ГЕПАТОПРОТЕКТОРНОЇ ДІЇ

О.Ю.Ткачук, Л.І.Вишневська, Т.М.Зубченко

***Ключові слова**: олійні екстракти; критичні параметри; технологія; композиція рослинної сировини; гепатопротектор*

Проведені експериментальні дослідження дали можливість визначити критичні параметри та їх вплив на технологічний процес виробництва олійних екстрактів композиції лікарської рослинної сировини гепатопротекторної дії. Вивчено вплив розміру частинок сировини,концентрації і кількості етанолу на зволожування рослинної композиції і температури екстракції. У результаті досліджень визначені оптимальні параметри екстракції олійними екстрагентами, які забезпечують ефективність вивільнення діючих речовин із рослинної сировини: подрібнення рослинної сировини з використанням шнекового подрібнювача, екстрагент для зволоження – 70% етанол у кількості 0,6±0,1 мл на 1,0 сировини; час зволоження – 2,0±0,5 год; температура екстракції – 55±5ºС. За визначеними критичними точками технологічного процесу розроблена технологічна схема виробництва комбінованого олійного фітопрепарату під умовною назвою «Олеосил».

ИЗУЧЕНИЕ ВЛИЯНИЯ КРИТИЧЕСКИХ ПАРАМЕТРОВ ПРОИЗВОДСТВА НА ТЕХНОЛОГИЧЕСКИЙ ПРОЦЕСС МАСЛЯНОГО ФИТОЭКСТРАКТА ГЕПАТОПРОТЕКТОРНОГО ДЕЙСТВИЯ

О.Ю.Ткачук, Л.И.Вишневская, Т.Н.Зубченко

***Ключевые слова:** масляные экстракты; критические параметры; технология; композиция растительного сырья; гепатопротектор*

Проведенные экспериментальные исследования дают возможность определить критические параметры и их влияние на технологический процесс производства масляных экстрактов композиции лекарственного растительного сырья гепатопротекторного действия. Изучено влияние размера частиц сырья, концентрации и количества этанола на увлажнение растительной композиции и температуры экстракции. В результате исследований определены оптимальные параметры экстракции масляными экстрагентами, которые обеспечивают эффективность высвобождения действующих веществ из растительного сырья: измельчение растительного сырья с использованием шнекового измельчителя, экстрагент для увлажнения – 70% этанол в количестве 0,6±0,2 мл на 1,0 сырья; время набухания – 2,0±0,5 ч; температура экстракции – 55±5°С. По определенным критическими точкам технологического процесса разработана технологическая схема производства комбинированного масляного фитопрепарата под условным названием «Олеосил».

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

UDC 615.281.8:54.057

"GREENING" OF AMIZONE SYNTHESIS WHEN MANUFACTURING

V.A.Georgiyants, V.M.Kushniruk, P.O.Bezugly

National University of Pharmacy

Farmak Joint-Stock Company

Key words: Amizone; manufacturing synthesis; "green chemistry"

The principles of "green chemistry" have been introduced into the procedure of manufacturing synthesis of Amizone. All starting raw materials as solvents have been assessed in accordance with their human toxicity, danger in production, as well as the possible impact on the environment and cost. According to the results of the analysis isonicotinic acid has been chosen as a starting substance for the synthesis at the first step, and the synthetic procedure without solvents has been proposed. The replacement of acetone with isopropanol has been proposed for the alkylation step according to the data concerning toxicity and manufacturing safety. Aqueous ethanol has been chosen as a solvent for recrystallisation. To reduce the impact on the environment the process of conversion of the benzylamine excess into less toxic and flammable hydrochloride has been recommended.

The quality of a pharmaceutical drug is affected by many factors. However, the main of them is the quality of its components, and above all – the quality of the active pharmaceutical ingredient/ingredients (APIs). For a long period manufacturers have had to choose for themselves the manufacturer of API with reliable results on the quality of the API. Today, the main suppliers of pharmaceutical substances are India and China. In some cases, they are the monopolists for production of certain substances.

Unfortunately, only some plants commercially produce APIs in Ukraine. They are, for example, "Farmak" JST, "InterChem" JLC PJSC, SIC "Borshchahivskiy CPP". As a rule, it is the synthesis of the original substances, which are manufacturers' "brands", for example "Thiotriazoline", "Amizone" and others. One of the problems associated with reduction of the API synthesis by industrial enterprises in developed countries is a difficult environmental situation and high requirements to the producers to waste and their control [7, 10, 11].

One of the ways to reduce the impact on the atmosphere during the process of API industrial synthesis is widespread adoption of the principles of "green chemistry" by the leading pharmaceutical companies in the world [5, 8, 9]. These principles can not only reduce the risk of the environmental pollution, but decrease the effect of hazardous reagents on the health of employees engaged in the API manufacture.

The aim of our work was to implement the principles of "green chemistry" into the industrial synthesis of Amizone by "Farmak" JST.

Materials and Methods

The synthesis of Amizone was carried out according to the general principles of organic synthesis. When preparing to the re-registration of Amizone substance associated with the organization of a new production site for the API synthesis in Shostka we carried out the preliminary experimental studies to improve the methods of synthesis allowing to increase the yield of the end product and decrease the amounts of related impurities [2, 3].

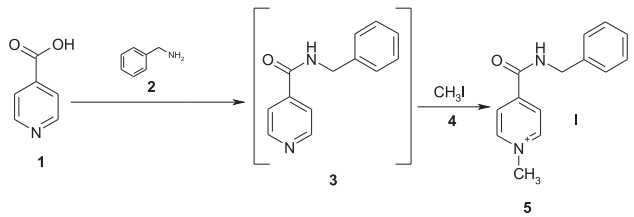
For evaluation the modern international classification – Globally Harmonized System of Classification and Labelling of Chemicals (GHS) [6] applied all over the world when working with chemicals was used. The relative cost of reagents was given according to the data of the Sigma-Aldrigh company.

Results and Discussion

As a result of our previous research [2, 3] we recommend to carry out the synthesis according to Scheme.

When introducing this Scheme into the industrial synthesis we paid attention to the maximum "greening" of the procedure developed. Due to the main chemical rules we can use different starting substances such as isonicotinic acid, its esters and chloranhydride and different solvents during both stages of synthesis. The principles of "green chemistry" include 12 basic ones [4]. We analyzed these principles and tried to assess the compliance of the new methodology, as well as offer additional improvements to meet these requirements.

Firstly, toxicity and properties of possible reagents and solvents (Tab. 1) were analysed. While developing the synthetic technology [2, 3] the choice of starting materials, mainly focusing on their cost and reaction yields, was evaluated. Due to the combination of "cost-yield-purity" parameters the advantage was given to the synthesis from isonicotinic acid. When studying the "greening" synthesis,



Scheme

Table 1

Information about reagents and solvents

Substance, CAS number	Toxicity (GHS Classification)	Hazard statement(s)	Cost/100 g
Isonicotinic acid 55-22-1	Acute toxicity, Oral (4) Skin irritation (2)	H302: Harmful if swallowed. H315: Causes the skin irritation.	36
Ethyl isonicotinate 1570-45-2	GHS – None found Xi – Irritant	H315: Causes the skin irritation. H319: Causes a serious eye irritation. H335: May cause the respiratory irritation.	63.10
Methyl isonicotinate	Flammable liquids (4) Skin irritation (2) Eye irritation (2A) Specific target organ toxicity – single exposure (3), Respiratory system	H315: Causes the skin irritation. H319: Causes a serious eye irritation. H335: May cause the respiratory irritation.	78.10
Isonicotinyl chloride	GHS – None found	No information	81.90*
Isonicotinyl chloride hydrochloride 39178-35-3	Skin corrosion (1B) Serious eye damage (1)	H314: Causes severe skin burns and eye damage.	381
Benzylamine 100-46-9 221-943-6	Flammable liquids (3) Skin corrosion/irritation (1) Serious eye damage/eye irritation (1) Health hazards not otherwise classified (corrosion) (1)	H314: Causes severe skin burns and eye damage. H318: Causes a serious eye damage. H226: Flammable liquid and vapour.	30.70
Benzylamine hydrochloride 3287-99-8	Acute toxicity, Oral (4) Skin irritation (2) Serious eye damage/eye irritation (2) Specific target organ systemic toxicity (3)	H302: Harmful if swallowed. H315: Causes the skin irritation. H318: Causes a serious eye damage. H335: May cause the respiratory irritation.	95.10
Iodomethane 74-88-4	Acute toxicity (Oral) (3) Acute toxicity (Inhalation: Vapours) (3) Skin corrosion/irritation (2) Serious eye damage/eye irritation (1) Specific target organ toxicity – Single exposure (3) (drowsiness and dizziness, respiratory irritation) Specific target organ toxicity – Repeated exposure (2) (thyroid gland, respiratory)	H301 + H331: Toxic if swallowed or if inhaled. H312: Harmful in contact with the skin. H315: Causes the skin irritation. H319: Causes a serious eye irritation. H335: May cause the respiratory irritation. H351: Suspected of causing cancer. H410: Very toxic to aquatic life with long lasting effects.	88
Acetone 67-64-1	Flammable liquids (2) Skin irritation (3) Eye irritation (2A) Specific target organ toxicity – single exposure (3)	H225: Highly flammable liquid and vapour. H316: Causes a mild skin irritation. H319: Causes a serious eye irritation. H336: May cause drowsiness or dizziness.	
Propanol-2 67-63-0	Flammable liquids (2) Eye irritation (2) Specific target organ toxicity – single exposure (3)	H225: Highly flammable liquid and vapour. H319: Causes a serious eye irritation. H336: May cause drowsiness or dizziness.	
Ethanol 64-17-5	Flammable liquids (2) Skin irritation (2) Eye irritation (2B) Specific target organ toxicity – single exposure (3) Acute aquatic toxicity (2)	H225: Highly flammable liquid and vapour. H315 + H320: Causes the skin and eye irritation. H335: May cause the respiratory irritation. H401: Toxic to aquatic life.	

* For 25 mg.

Table 2

The principles of "green chemistry" in the synthesis of Amizone

The "green chemistry" principles [4]	The synthetic steps and parameters controlled	Result and decision
It is better to prevent waste than to treat or clean up waste after it is formed.	Development of an optimal synthetic procedure.	Pharmacopoeial purity after recrystallization.
Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.	The total yield after all synthetic stages and recrystallization.	Intermediate yield – 92% End product yield – 87% After recrystallisation – 87%
Wherever practicable, synthetic methodologies should be designed to use and generate substances that possess little or no toxicity to human health and the environment.	The choice of the starting raw materials and solvents.	Safety class of the reagent is taken into account.
Chemical products should be designed to preserve efficacy of function while reducing toxicity.	The product is not novel.	–
The use of auxiliary substances (e.g. solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.	Decrease in use of auxiliary substances.	Development of the synthetic procedure without a solvent.
Energy requirements should be recognized for their environmental and economic impacts and should be minimized. Synthetic methods should be conducted at ambient temperature and pressure.	Development of the procedure at normal pressure and the minimal temperature.	Isonicotinic acid as a starting raw material is more economical; the synthesis without solvents at normal pressure (high temperature).
A raw material or feedstock should be renewable rather than depleting wherever technically and economically practicable.		Yields are very high. There is not need in reagents recycling. Acidifying of the uterine solution for benzylamine hydrochloride formation.
Reduce derivatives – Unnecessary derivatization (blocking group, protection/deprotection, temporary modification) should be avoided whenever possible.		An intermediate is not isolated, blocking groups are not used.
Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.	Studying the possibility and expediency of the use of catalysts in the synthesis.	The synthesis with high yields without any catalysts (the cost is reasonable).
Chemical products should be designed so that at the end of their function they do not persist in the environment and break down into innocuous degradation products.	Development of approaches to waste management.	Convertion of the benzylamine residue into less toxic hydrochloride.
Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.	Development of in-process monitoring procedures.	Chromatographic control of the benzylamine residue when manufacturing [1].
Substances and the form of the substance used in a chemical process should be chosen to minimize potential for chemical accidents, including releases, explosions, and fires.	Revision of the starting raw materials and the solvents according to their fire and explosion properties.	Replacing of isonicotinoyl chloride with the acid. Avoiding the use of acetone in the synthesis during amidation – carrying out the synthesis without solvents; replacing ethanol in recrystallisation processes by aqueous ethanol.

besides these factors, the attention was paid to the human toxicity and danger in production, as well as the possible impact on the environment.

Analyzing the data giving in Tab. 1 concerning toxicity of reagents and their cost it can be concluded that the best starting material is isonicotinic acid, and chloranhydride is unacceptable in manufacturing even when it is introduced into the reaction as a hydrochloride. Ethyl and methyl esters of isonicotinic acid are slightly more toxic; moreover, they are classified as potentially flammable liquids unlike isonicotinic acid.

The same principles were taken into account when analysing the choice of solvents for both synthesis and recrysrallisation. During our previous investigation it was found that the reaction of amination between isonicotinic acid and benzylamine could be carried out without any solvents. Previously the conditions for the best yields and optimal purity were discussed. Such procedure is consistent with the principles of "green chemistry". The next step of the synthesis is alkylation of the key intermediate – isonicotinic acid benzylamide with methyl iodide. There is no choice of reagents because of the structure

of the end product. The replacement of acetone with isopropanol is reasonable at this stage according to the data of toxicity and manufacturing safety (Tab. 1). Due to the "green chemistry" principles water is an optimal reagent for any process. When choosing a solvent for recrystallisation of Amizone in its manufacturing, unfortunately, the replacement of aqueous ethanol with water is not reasonable because it reduces purity and yields to the limit of pharmacopoeial requirements on the content of impurities [3].

In addition to achieving high yield of the synthesis development of methods for unreacted substances recycling is important to reduce the impact on the environment. Among reagents used for the synthesis of Amizone it is necessary to convert benzylamine into less toxic and flammable hydrochloride.

The summary of the steps for "greening" the synthesis of Amizone is given in Tab. 2.

CONCLUSIONS

The procedure for "green" manufacturing synthesis of Amizone has been developed. The use of the starting raw materials and solvents has been substantiated in accordance with their safety and toxicity, as well as recycling of residues of the reagents.

REFERENCES

1. *Георгиянц В.А., Кушнирук В.Н., Гарная Н.В. // Вестник Южно-Казахстанской фармацевтической академии. – 2014. – Т. 2, №3. – С. 27-30.*
2. *Георгиянц В.А., Кушнирук В.Н., Гарная Н.В. // Запорожский мед. журн. – 2014. – №4(85). – С. 86-89.*
3. *Кушнірук В.М., Георгіянц В.А., Гарна Н.В. // Фармац. часопис. – 2013. – №4. – С. 12-15.*
4. *Anastas P.T., Warner J.C. Green Chemistry: Theory and Practice, Oxford University Press. – New York, 1998. – P. 30.*
5. *Bing-Feng Sun // Tetrahedron Lett. – 2015. – Vol. 56, №17. – P. 2133-2140.*
6. *Globally Harmonized System of Classification and Labelling of Chemicals (GHS), United Nations Economic Commission for Europe. – 2007. – P. 839-924.*
7. *Haleema R.M., Salemb M.Y., Fatahallaha F.A., Abdelfattah L.E. // Saudi Pharmac. J. – 2015. – Vol. 23, №5. – P. 463-469.*
8. *Jolliffe H.G., Gerogiorgis D.I. // Chemical Engineering Res. and Design. – 2015. – Vol. 97, №5. – P. 175-191.*
9. *Rastogi T., Leder C., Kümmerer K. // Chemosphere. – 2014. – Vol. 111, №9. – P. 493-499.*
10. *Ur Rehmana M.S., Rashida N., Ashfaqb M. et al. // Chemosphere. – 2015. – Vol. 138, №11. – P. 1045-1055.*
11. *Velagaletia R., Burns P. // Particulate Sci. and Technol. – 2007. – Vol. 25, №2. – P. 117-127.*

«ОЗЕЛЕНЕННЯ» СИНТЕЗУ АМІЗОНУ В ПРОЦЕСІ ПРОМИСЛОВОГО ВИРОБНИЦТВА

В.А.Георгіянц, В.М.Кушнірук, П.О.Безуглий

***Ключові слова**: амізон; промисловий синтез; «зелена хімія»*

Принципи «зеленої хімії» впроваджені в процес промислового синтезу субстанції Амізон. Оцінювання всіх вихідних речовин та розчинників здійснювали у відповідності з їх токсичністю для людини, небезпекою у виробництві, а також можливим впливом на навколишнє середовище та вартістю. За результатами аналізу для синтезу на першій стадії як вихідний реагент було обрано ізонікотинову кислоту та запропонована синтетична методика без використання розчинників. У відповідності з даними про токсичність для стадії алкілування запропонована заміна ацетону ізопропанолом. Як розчинник для перекристалізації амізону-сирцю запропоновано водний етанол. Для зменшення впливу на навколишнє середовище рекомендовано перетворення надлишку бензиламіну на менш токсичний та займистий гідрохлорид.

«ОЗЕЛЕНЕНИЕ» СИНТЕЗА АМИЗОНА В ПРОЦЕССЕ ПРОМЫШЛЕННОГО ПРОИЗВОДСТВА

В.А.Георгиянц, В.Н.Кушнирук, П.А.Безуглый

***Ключевые слова:** амизон; промышленный синтез; «зеленая химия»*

Принципы «зеленой химии» внедрены в процесс промышленного синтеза субстанции Амизон. Оценивание всех исходных веществ и растворителей осуществляли в соответствии с их токсичностью для человека, опасностью в производстве, а также возможным влиянием на окружающую среду и стоимостью. По результатам анализа для синтеза на первой стадии в качестве исходного реагента была выбрана изоникотиновая кислота и предложена синтетическая методика без использования растворителей. В соответствии с данными о токсичности и для стадии алкилирования предложена замена ацетона изопропанолом. В качестве растворителя для перекристаллизации амизона-сырца предложен водный этанол. Для снижения влияния на окружающую среду рекомендовано превращение избытка бензиламина в менее токсичный и воспламеняющийся гидрохлорид.

ОРГАНІЗАЦІЯ ТА ЕКОНОМІКА ФАРМАЦІЇ

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

UDC 615.242:615.453:339.138:339.13.021

ANALYSIS OF OROMUCOSAL MEDICINAL PRODUCTS IN SOLID DOSAGE FORMS FOR THERAPEUTIC DENTISTRY

L.I.Shulga, K.A.Chikhladze, S.M.Rolik, S.O.Povetkin

National University of Pharmacy

Key words: dental medicinal products with the local action; solid dosage forms; marketing research; pharmaceutical market

In order to determine the necessity of creating dental medicinal products the oromucosal dosage forms of the Ukrainian pharmaceutical market have been analysed. Tablet drugs are increasingly used in the treatment of periodontal diseases. Based on the results of the analysis of 35 drugs it has been found that the share of drugs produced in Ukraine (23%) ranks second after Germany (31%) among manufacturer countries. According to the data of the marketing research 54.3% of solid dosage forms are tablets, 25.7% – lozenges and 20% – pastilles. It has been determined that 34.3% of drugs are two-component products, 25.7% – three-component, 20% are one-component and 20% are multi-component. Distinguishing the concept of active substances it has been shown that 77.1% of drugs contain antiseptics, 14.3% – antibiotics, 20% – essential oils, 17.1% – anesthetics. Natural substances as a component are in 8.6% of medicinal products, e.g. phenolic hydrophobic propolis preparation in "Proalor" (LLC "Pharmaceutical company "Zdorovie", Ukraine), a thick sage extract in "Shavlia" (Natur Product Europa BV, Netherlands) and a solid chlorophyllipt extract in "Chlorophyllipt" tablets (LLC "Pilot Plant "State Scientific Centre on Medicinal Products", Ukraine). It has been found that there is dominance of combined drugs and a small share of drugs based on natural substances; therefore, creation of oromucosal herbal medicinal products in a solid dosage form is expedient.

One of the most pressing problems in Ukraine is the health status of the population, including such component as dental health, which low level has a negative impact on the general health condition during all periods of life [4]. Dental service is growing rapidly in Ukraine. Physicians prescribe drugs to patients in different dosage forms for therapeutic purposes [5]. Scientific works of domestic and foreign researchers focus on pharmacotherapeutic issues of oral inflammatory diseases outlining the ways to create new drugs for dental practice, defining the prospects for new research based on the assessment of the range of dental drugs at the Ukrainian pharmaceutical market, etc. [6, 8, 11].

Dental medicinal products can be solid (tablets, lozenges, pastilles, herbal teas), soft (pastes, gels) or liquid (solutions for rinsing the mouth, tinctures) drugs.

Pharmacotherapy of inflammatory periodontal diseases increasingly offers solid dosage forms, such as orally disintegrating (mouth dissolving) tablets, sublingual tablets, lozenges or pastilles that are easy to administer and have pleasant organoleptic properties for a patient [7, 14]. Each of these solid dosage forms has its own specific characteristics. This is primarily due to the advantages in use, dosage of this form, the absence of difficulties with swallowing or discomfort of passage through the gastrointestinal tract, the possibility of combining several active substances with addition of flavouring agents to enhance organoleptic properties, and it is achieved by the technology of manufacturing the abovementioned medicinal form [3]. Oral pills are usually represented by uncoated tablets with the composition providing slow release and the local action of the active substance or substances, or release and absorption of the active substance or substances in certain parts of the mouth [9, 12, 13]. Lozenges and pastilles are solid single-dose drugs dissolving in the mouth for the local effect, containing one or more active ingredients; they are usually aromatic or sweet-based. They are prescribed for slow dissolution or dispersion in the oral cavity as a result of disintegration, i.e. they are at the site of inflammation for a long period of time, and it allows prolonging the local exposure of active substances [1].

The aim of the research is to carry out the comprehensive assessment of the range of solid drugs used in dental practice in treating diseases of the oral cavity and to determine the expedience of developing new dental products of this dosage form.

Materials and Methods

The study involved medicinal products for dental practice of the following groups according to the ATC classification [1]: AA01 – Stomatological preparations (A01A B53 – Chlorhexidine, combinations; A01A D – Other agents for

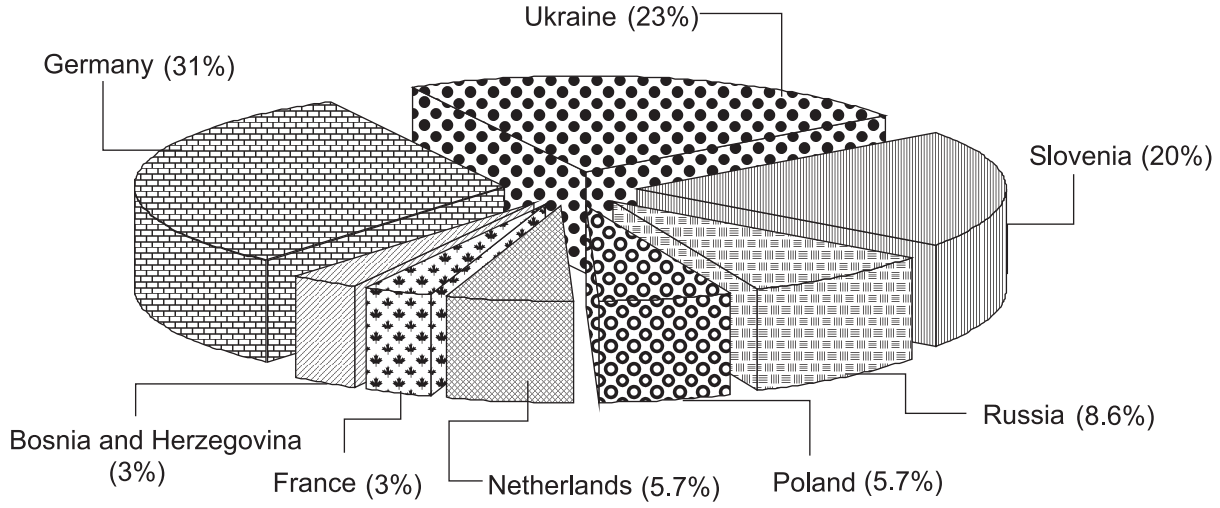


Fig. 1. Manufacturer countries of medicinal products for dentistry in solid dosage forms.

local oral treatment, including group A01A D11 – others); R02 A – Throat preparations (R02A A – Antiseptics: R02A A05 – Chlorhexidine, R02A A06 – Cetylpyridinium, R02A A020 – Various antiseptics, R02A A50** – Chlorhexidine, combinations; R02A B – Antibiotics: R02A B30 – Gramicidin, R02A B52 – Tyrothricin, R02A B53** – Other preparations) and D08 – Antiseptics and disinfectants (D08A X – Other antiseptics and disinfectants: D08A X10 – other preparations).

Using the concentric method the information search was conducted, and 35 drugs labeled for "inflammation of gums and oral mucosa, periodontitis, stomatitis, gingivitis" in the solid dosage form were analysed [2].

Results and Discussion

A detailed analysis of drug manufacturers has shown that Ukraine as a supplier of medicinal products for dental practice ranks second after Germany since the number of solid dosage forms produced by this country amounts for almost one third (31%) of the total amount. The rest of drugs are supplied by such manufacturer countries as Slovenia, Russia, Poland, Netherlands, France and Bosnia and Herzegovina. It is shown in Fig. 1.

Fig. 2 shows distribution of solid drugs in therapeutic dentistry. It has been determined that among the medicinal products studied pills (including orally disintegrating tablets, oral tablets) are 54.3%, lozenges – 25.7% and pastilles – 20%.

A separate study was performed to assess the amount of active drug substances: 34.3% of the segment under research belonged to two-component drugs, 25.7% – three-component, 20% – one-component and 20% – multi-component. Most of the drugs are combined (Fig. 3). They comprise several antimicrobial components with different mechanisms of action and include the following combinations: antiseptic + vitamin, antiseptic + anesthetic, antiseptic + vitamin + anesthetic, antibacterial component + antiseptic, antiseptic + essential oils, antiseptic + extracts of medicinal plants, products of the natural origin + vitamin.

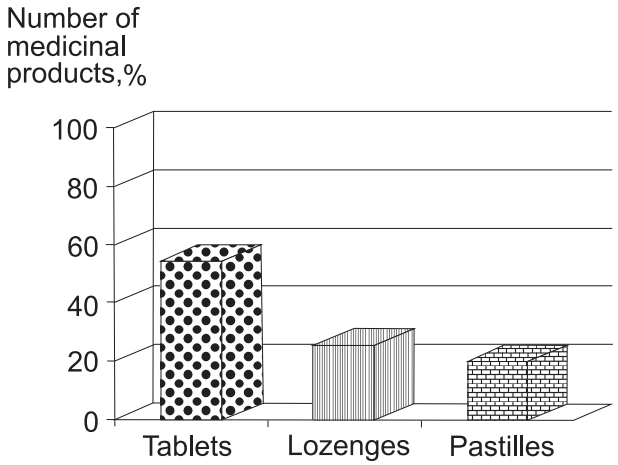


Fig. 2. The types of solid dosage medicinal products for therapeutic dentistry.

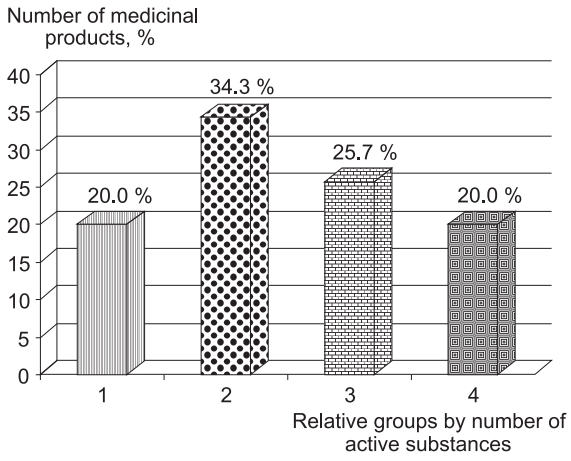


Fig. 3. The number of components in solid medicinal products used for treating inflammatory periodontal diseases, where: 1-4 – groups by the number of active substances. 1 – one-component drugs; 2 – two-component drugs; 3 – three-component drugs; 4 – multi-component drugs.

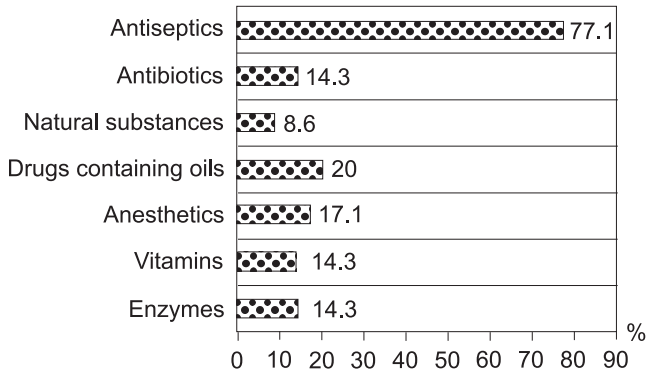


Fig. 4. The active components of solid medicinal products for dental practice.

Such combinations allow obtaining the desired therapeutic effect. Addition of various components of essential oils (menthol, anethole, thymol, etc.) to the antimicrobial component gives a mild local anaesthetic effect, while essential oils or extracts of medicinal plants (peppermint, sage, eucalyptus) enhance the antimicrobial and anti-inflammatory effect.

Medicinal products with a strong anesthetic action contain local anesthetics (benzocaine hydrochloride, lidocaine hydrochloride, oxybuprocaine chloride, etc.). Drugs for ulcerative necrotic lesions of the oral mucosa are found to contain enzyme components, such as lysozyme hydrochloride with antibacterial properties and ability to stimulate the body's non-specific reactivity by splitting necrotic tissues.

The total number of drugs includes 77.1% of drugs with antiseptics as the main components (Fig. 4), namely chlorhexidine, dichlorbenzyl alcohol, cetylpyridinium, decamethoxin, dequalinium, benzalkonium, amylmetacresol, biclotymol. Some drugs (Neo-angin® sugar-free, Neo-angin, Neo-angin® Sage, Neo-angin® Cherry (Divapharma GmbH Klosterfrau Berlin GmbH, Germany) combine several antiseptics, such as amylmetacresol and dichlorbenzyl alcohol. Antibiotics are components of 14.3% of drugs. These are such active ingredients as tyrothrisin Trachisan (Engelhard Arzneimittel GmbH & Co.KG, Germany), dorithricin (RIEMSER Specialty Production GmbH, Germany) and gramicidin (Grammidin® with anesthetic Neo, Grammidin® Neo) and Grammidin® (JSC "Valenta Pharmaceuticals", Russian Federation).

Natural substances as a component are in 8.6% of medicinal products, e.g. phenolic hydrophobic propolis preparation in "Proalor" (LLC "Pharmaceutical company "Zdorovie", Ukraine), a thick sage extract in "Shavlia" (Natur Product Europa BV, Netherlands) and a solid chlorophyllipt extract in "Chlorophyllipt" tablets (LLC "Pilot Plant "State Scientific Centre on Medicinal Products", Ukraine).

The antibacterial agent and lysozyme are contained in 14.3% of drugs (Hexalyse (Laboratoires BOUCHARA-RECORDATI, France) Lysobact® (Bosnalijek dd, Bosnia and Herzegovina), Lizak® (3 names) JSC "Farmak", Ukraine), and 14.3% of drugs, e.g. (Septalor (LLC "Ternopharm", Ukraine), Lysobact (Bosnalijek dd, Bosnia and Herzegovina), Proalor (LLC "Pharmaceutical company "Zdorovie", Ukraine), Sebidin and Sebidin Plus (Glaxo SmithKline, Poland), etc., have vitamin components (ascorbic acid and pyridoxine hydrochloride) in their composition. More than 50% of the abovementioned drugs contain ascorbic acid in combination with chlorhexidine. It has been noted that 20% of drugs combine components of essential oils (menthol, levomenthol, anethole, thymol) or essential oils themselves (peppermint oil, eucalyptus oil) with antiseptics (benzalkonium chloride – Septolete, Septolete D (KRKA, Slovenia), dichlorbenzyl alcohol – Angi-Sept (5 names) Dr. Theiss Naturwaren GmbH, Germany).

The anesthetic component (active substances – benzocaine, oxybuprocaine chloride, tetracaine hydrochloride, lidocaine hydrochloride) are in 17.1% of the drugs under study (Septolete® Plus Menthol, Septolete® Plus Honey and Lime (KRKA, Slovenia) Anti-Angin Formula (HERKEL BV, Netherlands), Grammidin® with anesthetic Neo (JSC "Valenta Pharmaceuticals", Russian Federation) Trachisan (Engelhard Arzneimittel GmbH & Co.KG, Germany), Dorithricin (RIEMSER Specialty Production GmbH, Germany)).

CONCLUSIONS

The range of solid medicinal products that are available at the domestic pharmaceutical market and used in dental practice in the treatment of inflammatory periodontal disorders and oral diseases has been analysed.

Medicinal products have been characterized depending on the manufacturer country, the type of a solid dosage form and structured according to the amount and content of active components and their combination.

It has been found that the segment of natural oromucosal drugs for therapeutic dentistry is too small.

It has been determined that creation of a new oromucosal herbal medicinal product in a solid dosage form is based on natural substances can be considered to be a relevant and up-to-date task for pharmaceutical technology.

REFERENCES

1. *АТС-класифікація лікарських засобів [http://www.compendium.com.ua].*
2. *Державний реєстр лікарських засобів України [http://www.drlz.kiev.ua].*
3. *Могилюк В.В. // Провізор. – 2009. – №1-2. – С. 32-35.*
4. *Павленко О.В., Савичук Н.О., Драгомирецька М.С. та ін. // Інноваційні технології – в стоматологічну практику: Матер. III (X) з'їзду асоціації стоматологів України, м. Полтава, 16-18 жовтня 2008 р. – Полтава: Дивосвіт, 2008. – С. 37-41.*
5. *Шульга Л.І., Безценна Т.С., Пімінов О.Ф. та ін. // Запорожский мед. журн. – 2012. – №5. – С. 110-113.*

6. *Шульга Л.І. // Клінічна фармація, фармакотерапія та медична стандартизація. – 2011. – №3-4. – С. 152-156.*
7. *Al-Khattawi A., Mohammed A. // Expert Opin. Drug Deliv. – 2013. – Vol. 10, №5. – P. 651-663.*
8. *Bascones-Martínez A., Eignero Ruiz E. // Advances an Periodoncia (Av Periodontal Implantol.). – 2005. – Vol. 3. – P. 111-118.*
9. *Birudaraj R., Mahalingam R., Li X. et al. // Crit. Rev. Ther. Drug Carrier Syst. – 2005. – Vol. 22, №3. – P. 295-330.*
10. *Encyclopedia of Pharmaceutical Technology / Ed by J.Swarbrick. – 3-rd ed. – NY: Informa Healthcare USA, Inc., 2007. – 4372 p.*
11. *Jothi V., Vijay K.T., Vasudev B. et al. // Advances in Biosci. and Biotechnol. – 2013. – Vol. 4. – P. 15-18.*
12. *Perioli L., Ambrogi V., Giovagnoli S. et al. // AAPS PharmSciTech. – 2007. – Vol. 8, №3. – P. 54.*
13. *Zhang D., Liu C., Zhang J. et al. // J. of Southern Med. University. – 2014. – Vol. 34, №9. – P. 1386-1389.*
14. *Wennström J.L. // J. of Periodontol. – 2014. – Vol. 85, №12. – P. 1639-1641.*

АНАЛІЗ ОРОМУКОЗНИХ ПРЕПАРАТІВ ДЛЯ ТЕРАПЕВТИЧНОЇ СТОМАТОЛОГІЇ У ВИГЛЯДІ ТВЕРДИХ ФОРМОВАНИХ ЛІКАРСЬКИХ ФОРМ

Л.І.Шульга, К.А.Чіхладзе, С.М.Ролік, С.О.Повєткін

***Ключові слова**: стоматологічні препарати місцевої дії; тверді лікарські форми; маркетингові дослідження; фармацевтичний ринок*

З метою визначення необхідності створення стоматологічних лікарських засобів (ЛЗ) проаналізовані оромукозні лікарські форми (ЛФ) фармацевтичного ринку України. При лікуванні захворювань пародонту дедалі частіше застосовують таблетовані ЛЗ. За результатами аналізу 35 ЛЗ зазначеного сегменту встановлено, що частка препаратів вітчизняного виробника (23%) поступається серед країн-виробників лише Німеччині (31%). Маркетинговим аналізом твердих формованих ЛЗ визначено, що 54,3% – це таблетки, 25,7% – льодяники, 20% – пастилки. Підраховано, що за кількістю компонентів 34,3% ЛЗ є двокомпонентними, 25,7% – трикомпонентні, по 20% – одно- та багатокомпонентні препарати. Розмежовуючи поняття про діючі субстанції, ми виявили, що антисептична складова зустрічається у 77,1% препаратів, антибіотики – у 14,3%, ефірні олії – у 20%, анестетики – у 17,1%. Природні субстанції є у складі у 8,6% ЛЗ. Це фенольний гідрофобний препарат прополісу у ЛЗ «Проалор» (ТОВ «Фармацевтична компанія «Здоров'я», Україна), екстракт шавлії сухий у ЛЗ «Шавлія» (Natur Product Europa B.V., Нідерланди) та хлорофіліпту екстракт густий у складі таблеток «Хлорофіліпт» (ТОВ «Дослідний завод «ДНЦЛЗ», Україна). Вивченням підкреслено домінування комбінованих ЛЗ і замалу частку ЛЗ на основі природних субстанцій, що підтверджує доцільність створення оромукозних рослинних ЛЗ у вигляді твердих ЛФ для стоматологічної практики.

АНАЛИЗ ОРОМУКОЗНЫХ ПРЕПАРАТОВ ДЛЯ ТЕРАПЕВТИЧЕСКОЙ СТОМАТОЛОГИИ В ВИДЕ ТВЕРДЫХ ФОРМИРУЕМЫХ ЛЕКАРСТВЕННЫХ ФОРМ

Л.И.Шульга, Е.А.Чихладзе, С.Н.Ролик, С.А.Поветкин

***Ключевые слова**: стоматологические препараты местного действия; твердые лекарственные формы; маркетинговые исследования; фармацевтический рынок*

С целью определения необходимости создания стоматологических лекарственных средств (ЛС) проанализированы оромукозные лекарственные формы (ЛФ) фармацевтического рынка Украины. При лечении заболеваний пародонта все чаще применяют таблетированные ЛС. По результатам анализа 35 ЛС вышеупомянутого направления установлено, что доля препаратов отечественного производителя (23%) уступает среди стран-производителей только Германии (31%). Маркетинговым анализом твердых формируемых ЛС отмечено, что 54,3% – это таблетки, 25,7% – леденцы, 20% – пастилки. Подсчитано, что по количеству компонентов 34,3% ЛС являются двухкомпонентными, 25,7% – трехкомпонентные, по 20% – одно- и многокомпонентные препараты. Разграничивая понятия о действующих субстанциях, выявлено, что антисептическая составляющая встречается у 77,1% препаратов, антибиотики – у 14,3%, эфирные масла – у 20%, анестетики – у 17,1%. Природные субстанции есть в составе 8,57% ЛС. Это фенольный гидрофобный препарат прополиса в ЛС «Проалор» (ООО «Фармацевтическая компания «Здоровье», Украина), экстракт шалфея сухой в ЛС «Шалфей» (Natur Product Europa B.V., Нидерланды) и хлорофиллипта экстракт густой в составе таблеток «Хлорофиллипт» (ООО «Опытный завод «ГНЦЛС», Украина). Изучением подчеркнуто доминирование комбинированных ЛС и малая доля ЛС на основе природных субстанций, что подтверждает целесообразность создания оромукозных растительных ЛС в виде твердых ЛФ для стоматологической практики.

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

UDC 615.074:612.63

ANALYSIS OF THE CAR FIRST AID KITS OF UKRAINE AND GREAT BRITAIN ACCORDING TO THE NORMATIVE DOCUMENTS

T.V.Diadiun, S.O.Mamedova

National University of Pharmacy

Key words: car first aid kit; first aid; labelling; normative documents

Many deaths in consequence of road traffic accidents could be prevented if first aid was given to the victim prior to the arrival of ambulances. This will require the presence of a first aid kit in a vehicle, the contents of which can provide aid in case of road traffic accidents and other injuries. First aid is the aid provided to the person, who suffered from sudden injury or illness. It includes a set of skills that have theoretical support and require some training. Today there are two regulations such as Order No. 187 and DSTU 3961-2000, and there are differences in the contents of the car first aid kit in Ukraine. The normative documents have been studied, and the difference between the contents of the car first aid kits in Ukraine and Great Britain has been determined. A comparative analysis has shown that citizens of Ukraine are more prepared for adverse situations that may arise in case of a road traffic accident. It is shown by the presence of drugs needed in case of emergency, while the British first aid kit contains mostly plasters and bandages. It has been also found that there is a difference in labelling of storage cases.

Every year hundreds of thousands of people are seriously injured or killed in consequence of road traffic accidents (RTA). Taking into account the increasing number of vehicles such statistics in the future will increase [7, 10]. To prevent this, the General Assembly of UN (dated 02.03.2010) adopted the resolution, which declared the years of 2011-2020 as Decade of Action on Road Safety [6]. It should be noted that many deaths in consequence of road traffic accidents could be prevented if first aid was given to the victim prior to the arrival of ambulances. This will require the presence of a first aid kit in a vehicle, the contents of which can provide aid in case of road traffic accidents and other injuries.

First aid is the aid provided to the person, who suffered from sudden injury or illness. It includes a set of skills that have theoretical support and require some training.

For this purpose the British Red Cross, for example, offers video training sessions on first aid in situations such as heavy bleeding, heart attack, fractures and many others. In training on first aid the teams of St John's Ambulance help to the British. They have a wide variety of training courses, including many sections, throughout the country.

There are countries where one must have the car first aid kit by law, e.g. in Austria, Croatia, Great Britain, and there are countries where its presence in the car is not regulated by law, but highly recommended, e.g. in Belgium, Denmark, and Germany.

The aim of the work was to carry out analysis of normative documents regulating completeness of the car first aid kit in Ukraine and Great Britain, compare and characterize their contents.

Materials and Methods

The contents of the car first aid kit for compliance with the Order of the Ministry of Public Health of Ukraine No. 187 dated 07.07.1998 and DSTU 3961-2000 dated 01.07.2000 "Car first aid kit. General requirements" and the EU kit with the contents regulated by the standard DIN13164 dated 01.01.1999, including the car first aid kit of Great Britain by the standard BS8599-2 dated 01.02.2014 have been studied [3, 5, 12].

Results and Discussion

The car first aid kit is a complex of medical devices used to provide first aid to victims of the accident and in the current operation. The list of the contents of the car first aid kit in Ukraine is given in Tab. 1 [4].

According to the Order No. 187 there are two types of kits: car kit-1 – for passenger cars and trucks (up to 9 passengers); car kit-2 – for passenger vehicles – buses (the carriage of more than 9 passengers) [5].

Car first aid kits are manufactured according to DSTU 3961-2000. It should be noted that currently, in the Order No. 187 and DSTU 3961-2000, there are differences in the contents of the car first aid kit regarding the availability of nitroglycerin, bactericidal plasters, scissors, gloves, pins and the amounts of butorphanol tartrate, which are not regulated at the state level [1].

Kits must be enclosed in separate sealed plastic bags, which have an information sticker indicating the purpose of the car first aid kit (car first aid kit-1 or car first aid kit-2); number of the kit, medical products included in the bag, and their number [3].

The state register of medical equipment and medical products includes several producers of car first aid kits:

- Private Joint-Stock company "Viola" pharmaceutical factory, Ukraine;
- Private Joint-Stock company "AV-Farma", Ukraine;
- Limited liability company "Kyiv Pharmaceutical Society", Ukraine;
- Private Joint-Stock company "Eximed", Ukraine [2].

Table 1

The list of the contents of the car first aid kit in Ukraine

Group	Name	Number	Purpose
Medical products for bleeding control and applying a bandage in injuries	Arresting bleeding tourniquet	1	Applied for temporary hemostasis from vessels in the limbs in the case if the bleeding can not be stopped in other ways
	Sterile bandage, 5 m×10 cm	1	Used for bandaging, fixation of wipes
	Wipes with chlorhexidine, 6×10 cm	2	Applied on an open wound as a pain reliever, have the antiseptic effect
	Styptic wipes with Furaginum 6×10 cm	2	Applied on an open wound as a pain reliever, have a pronounced hemostatic effect
	Sterile dressing pack	1	Consists of a gauze bandage, gauze and cotton swabs. Used for bleeding control
	Plaster in a roll 5 cm×5 m	1	Used for fixation of wipes
	Bactericidal plaster 2.3×7.2 cm	4	Applied in minor skin injuries
	Medical dressing kerchief 50×50 cm	1	Used for fixation of the extremities for fractures, dislocations, as well as for bandaging and for bleeding control
Antiseptics	5% Solution of iodine – 10 ml	1	Provides the antimicrobial and anti-inflammatory action
Analgesics and cardiac medicines	0.2% Butorphanol tartrate – 1 ml in a unit-dose syringe	2	Synthetic opioid analgesic. Effectively prevents a painful shock after injury
	Nitroglycerin 1% in capsules (0.0005) 20 tablets	1	Provides the vasodilatory effect, and is mostly used for relieving pain during angina attacks
Additional medical products	Scissors with blunt ends	1	Used for cutting bandages in the process of bandaging
	Medical gloves No. 8 of polyethylene	1	Used for short time protection of the skin from the aggressive environment
	Film (valve) for artificial ventilation of lungs	1	Used to protect the rescuer and the casualty when carrying out artificial lung ventilation by the "mouth-to-mouth method." Helps to avoid direct contact with mouth, nose (saliva or blood) of the victim
	20% Sulfacyl sodium – 1 ml in a unit-dose syringe	2	Eye drops. Applied to disinfect eyes if they are dirty
	English pins	6	Used for fixation of bandages, kerchief
	Instructions for the car first aid use	1	Used for available and comprehensive information about the application of each component of the kit
	A case for the first aid kit	1	Used for storage

Manufacturers offer two types of packaging for kits: a fabric case (cotton satin weave) or a plastic case.

The car kits under study are marked by these manufacturers and contain information about the purpose (car first aid kit-1, car first aid kit-2); they are not damaged and are fully packaged. Of all the drugs that are in the car kit butorphanol tartrate, nitroglycerin and solution of iodine have shorter shelf life. Because of this fact the recommended shelf life of car kits is 2 years. Such kit components as bandage, tourniquet, dressing kerchief have the longest shelf life – up to five years. Therefore, it is recommended to renew promptly or replace medicines after use or expiration.

In the UK the content of the car first aid kits is regulated by the British standard – BS8599-2 developed by the British Standards Institute (BSI) in 2014. The new national standard for first aid in cars became effective from February of 2014. This document aims to improve safety for all motorists in the UK.

Standard BS 8599-2 specifies the requirements to the content of components of the car kit and is presented in three sizes (small, medium, large). The size of the car kit required for a certain type of a motor vehicle is determined by the size and number of passengers. A small kit BS8599-2 is for moped, motorcycle and all-terrain vehicle (ATV) (maximum 3 passengers). The medium

kit BS8599-2 is for cars, taxis, vans and trucks (up to 8 passengers). The large kit BS8599-2 is for vans (maximum 16 passengers). If the number of passengers exceeds 17 or more, it is recommended to have two large kits BS8599-2. The list of the contents of the car kit in the UK is given in Tab. 2 [12].

There are several rules for labelling. It is allowed to put a white cross on a green background, or a green cross on a white background on the kit. The International Organization for Standards adopted this standard to facilitate the search of the kit for anyone who needs first aid. Sometimes kits are marked by a red cross on a white background, but the use of this emblem is permitted only to the International Committee of the Red Cross [11]. Otherwise it is considered as a violation of the terms of the First Geneva Convention adopted by the European Union.

CONCLUSIONS

Today there are two regulations such as Order No. 187 and DSTU 3961-2000, and there are differences in the contents of the car first aid kit in Ukraine. The normative documents have been studied, and the difference between the contents of the car first aid kits in Ukraine and Great Britain has been determined. A comparative analysis has shown that citizens of Ukraine are more prepared for adverse situations that may arise in case of a road traffic accident. It is shown by the presence of drugs needed in case of emergency, while the British first aid kit contains mostly plasters and bandages. It has been also found that there is a difference in labelling of storage cases.

Table 2

The list of contents of the car first aid kit in the UK

Contents	Small	Medium	Large
Instructions for use	1	1	1
Medium trauma dressing	1	1	2
Large trauma dressing	–	–	1
Triangular kerchief	–	1	2
Bactericidal plaster	5	10	20
Plaster in a roll	–	1	2
Sterile wet wipes	5	10	20
Sterile dressing	–	1	2
Medical gloves – pair	1	2	5
Face shield	1	1	2
Foil blanket	–	1	3
Burn dressing 10×10 cm	1	2	4
Clothing shears	1	1	1

REFERENCES

1. *Антимонопольний комітет зініціював зменшення витрат водіїв на автомобільні аптечки [Електронний ресурс]. – Режим доступу: http://www.amc.gov.ua/amc/control/uk/publish/article?art_id=158139&cat_id=142558.*
2. *Державний реєстр медичної техніки та виробів медичного призначення [Електронний ресурс]. – Режим доступу: http://portal.diklz.gov.ua/PublicSite/PUB/VMList.aspx.*
3. *ДСТУ 3961-2000. Аптечка медична автомобільна. Загальні вимоги (витяг) [Електронний ресурс]. – Режим доступу: http://www.infocar.com.ua/law_ukr/law_50.html.*
4. *Компендиум 2011 – лекарственные препараты: Справ. / Под ред. В.Н.Коваленко, А.П.Викторова. – К.: МОРИОН, 2011. – 2320 с.*
5. *Наказ МОЗ України №187 від 07.07.1998 р. «Про затвердження переліків лікарських засобів у медичних аптечках транспортних засобів» [Електронний ресурс]. – Режим доступу: http://zakon.rada.gov.ua/cgi-bin/laws/main.cgi?nreg=z0465-98.*
6. *Приєднуйтесь до десятиліття дій з безпеки дорожнього руху [Електронний ресурс]. – Режим доступу: http://roadsafety.org.ua/index.php?option=com_content&view=article&id=759%3A2011-04-21-10-10-21&cat id=65%3A2010-03-26-13-47-34&lang=uk.*
7. *Altenburger K. // Brandhilfe. – 1996. – №6. – S. 180.*
8. *Bahloul F.L., Gall J.R., Loirad R. et al. // Press Med. – 1988. – Vol. 17, №34. – P. 1741-1744.*
9. *Baker S.P. // New Enyl. J. Nod. – 1987. – №22. – P. 1384-1387.*
10. *Bull J.P. // Accident analysis and prevention. – 1973. – Vol. 5. – P. 45-53.*
11. *Geneva Convention (I) on 12 August 1949. "For the amelioration of the condition of the wounded and sick in armed forces in the field" [Електронний ресурс]. – Режим доступу: http://www.icrc.org/ihl.nsf/FULL/365?OpenDocument.*
12. *First Aid Kit Contents British Standard [Електронний ресурс]. – Режим доступу: http://evaq8.co.uk/First-Aid-Kit-Contents-British-Standard.html.*

АНАЛІЗ АПТЕЧКИ МЕДИЧНОЇ АВТОМОБІЛЬНОЇ УКРАЇНИ ТА ВЕЛИКОЇ БРИТАНІЇ ВІДПОВІДНО ДО НОРМАТИВНОЇ ДОКУМЕНТАЦІЇ

Т.В.Дядюн, С.О.Мамедова

***Ключові слова**: аптечка медична автомобільна; перша медична допомога; маркування; нормативна документація*

Багатьом випадкам загибелі в результаті ДТП можна було б запобігти, якби перша медична допомога потерпілому надавалась ще до прибуття карети швидкої допомоги. Для цього необхідною є наявність у транспортному засобі аптечки медичної автомобільної, вміст якої дозволяє надавати допомогу як у разі ДТП, так і при отриманні травм в інших випадках. Перша медична допомога (ПМД) – це допомога, що надається людині, яка постраждала від раптової травми або хвороби. ПМД включає в себе комплекс навичок, які мають теоретичне підкріплення і вимагають певної підготовки. Встановлено наявність двох нормативних документів, таких як Наказ №187 та ДСТУ 3961-2000, в яких існують розбіжності щодо вмісту автомобільної аптечки в Україні. Досліджено нормативну документацію та встановлено відмінність вмісту автомобільних медичних аптечок України та Великої Британії. Порівняльний аналіз показав, що громадяни України більш підготовлені до несприятливих ситуацій, які можуть виникнути в разі ДТП. Про це свідчить наявність препаратів, необхідних в екстрених випадках, в той час як британська аптечка наповнена здебільшого пластирами та пов'язками. Також встановлена відмінність у маркуванні футлярів для зберігання.

АНАЛИЗ АПТЕЧКИ МЕДИЦИНСКОЙ АВТОМОБИЛЬНОЙ УКРАИНЫ И ВЕЛИКОБРИТАНИИ СОГЛАСНО НОРМАТИВНОЙ ДОКУМЕНТАЦИИ

Т.В.Дядюн, С.А.Мамедова

***Ключевые слова**: аптечка медицинская автомобильная; первая медицинская помощь; маркировка; нормативная документація*

Многих случаев гибели в результате ДТП можно было бы избежать, если бы первая медицинская помощь потерпевшему оказывалась еще до прибытия кареты скорой помощи. Для этого необходимым является наличие в транспортном средстве аптечки медицинской автомобильной, состав которой позволяет оказывать помощь как в случае ДТП, так и при получении травм в иных случаях. Первая медицинская помощь (ПМД) – это помощь, которая предоставляется человеку, который пострадал от внезапной травмы или болезни. ПМД включает в себя комплекс навыков, которые имеют теоретическое подкрепление и требуют определенной подготовки. Установлено наличие двух нормативных документов, таких как Приказ №187 и ДСТУ 3961-2000, в которых существуют расхождения в содержимом автомобильной аптечки в Украине. Исследована нормативная документация и установлено отличие содержимого автомобильных медицинских аптечек Украины и Великой Британии. Сравнительный анализ показал, что граждане Украины более подготовлены к неблагоприятным ситуациям, которые могут возникнуть в случае ДТП. Об этом свидетельствует наличие препаратов, необходимых в экстренных случаях, в то время как британская аптечка содержит в основном пластыри и повязки. Также установлено отличие в маркировке футляров для хранения.

ЕКСПЕРИМЕНТАЛЬНА ТА КЛІНІЧНА ФАРМАКОЛОГІЯ

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

UDC 615.076:001.891.5:615.213/.214:547.856:547.583.5

THE PHARMACOLOGICAL ACTIVITY OF DERIVATIVES OF 4-OXO-3,4-DIHYDROQUINAZOLINE AND ANTHRANILAMIDES CONTAINING A FRAGMENT OF GLYCINE

Yu.O.Ovsyanikova, D.V.Levashov, V.M.Kravchenko, V.P.Chernykh, L.A.Shemchuk

National University of Pharmacy

Key words: quinazolone; anthranilamide; antidepressant effect; anticonvulsant activity; hypnotic activity

Derivatives of 4-oxo-3,4-dihydroquinazoline are known as a promising class of compounds due to their wide spectrum of the pharmacological activity. Taking into account the PASS data for the substituted anthranilamides synthesized, as well as derivatives of 4-oxo-3,4-dihydroquinazoline containing an "in-built" fragment of amino acid glycine as a pharmacophore, the decision to study their central neurotropic effects was made. While studying the hypnotic, anticonvulsant and antidepressant activities the highest antidepressant properties of N-(1,1-diphenyl-1-hydroxyet-2-yl)-N'-diphenylhydroxyacetylanthranilamide (compound 4) have been determined, this compound is slightly inferior the reference drug Imipramine. N-(phenylhydrazidoacetyl)-N'-succinamidoanthranilamide (compound 1) reveals high anticonvulsant properties and is not inferior the classical anticonvulsant drug Depakine. When studying the hypnotic effect the antagonism with Barbamyl for 2-(4-oxo-3,4-dihydro-3-quinazolinyl)acetohydrazide (compound 5) has been found. Methyl-(2-methylcarbonyloxymethyl-4-oxo-3,4-dihydro-3-quinazolinyl)acetate (compound 8) decreased the latent period of "falling asleep" for animals in 1.6 and 1.7 times in the doses of 20 and 200 mg/kg, respectively (the same level with the reference drug), and therefore, it is a promising compound for further research of the hypnotic activity. The analysis of the "structure-activity" relationship gives the possibility to assume that such pronounced pharmacological activity is due to the presence of substituents in position 2 of the quinazoline nucleus. Therefore, the data obtained prove that the study of these derivatives is promising for further search of new biologically active substances with hypnotic, anticonvulsant and antidepressant properties.

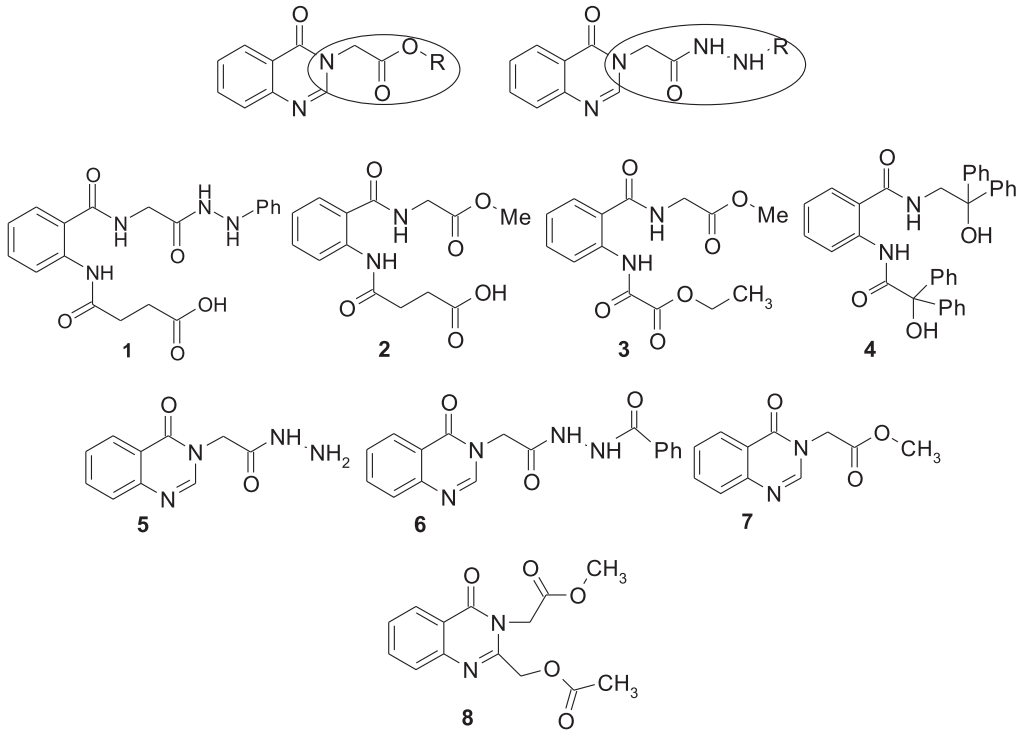
Derivatives of 4-oxo-3,4-dihydroquinazoline are known as a promising class of compounds due to their wide spectrum of the pharmacological activity. In particular, depending on the substituents in the quinazoline nucleus derivatives of this heterocyclic structure reveal the hypnotic, anticonvulsant, antibacterial, anticholinesterase, vasodilating activities [3, 4, 6, 9, 10]. Previously, derivatives of quinazoline and substituted anthranilamides (used as starting materials for quinazoline synthesis) containing an "in-built" fragment of amino acid glycine as a pharmacophore were synthesized [7, 8] (Fig.).

According to the data of the PASS software for compounds **1-8** the determination of some types of the pharmacological activity with probability higher than 0.75 was expected. Among them central neurotropic effects, namely antidepressant and anticonvulsant effects (for anthranilamides **1-4**) and hypnotic effect (for quinazolines **5-8**) prevailed. Therefore, the aim of this study was to conduct the pharmacological research of derivatives of 4-oxo-3,4-dihydroquinazoline and starting anthranilamides *in vivo*.

Materials and Methods

The antidepressant activity of compounds **1-4** was determined in white mice. For the depressive behaviour simulation the Porsolt behavioural despair test was used [5]. The test substances were injected intragastrically as water suspensions in the doses of 20 and 200 mg/kg 30 min prior to the experiment. Melipramin (Imipramine) was selected as a reference drug. It was injected intraperitoneally in the dose of 25 mg/kg. The control group received intragastrically the same volume of purified water. The total time of animal's immobile fixation and the number of immobility acts observed for 6 min were used as indicators of the antidepressant activity. The results obtained are given in Tab. 1.

The anticonvulsant activity was determined in white mice under conditions of experimental pentylenetetrazol convulsions [1]. The test substances were injected intragastrically as water suspensions in the doses of 20 and 200 mg/kg 30 min prior to the experiment. Depakine (sodium valproate) was selected as a reference drug.



Figure

Table 1

The effect of the test substances on the depressive behaviour of mice in the fixation test

Group of animals / test compound	Dose, mg/kg	Duration of immobile fixation, s	Number of immobility acts
Control	-	114.14+/-16.06	19.71+/-3.01
Compound **1**	20	71.20+/-10.26	15.40+/-1.40
	200	120.80+/-59.56	17.00+/-3.15
Compound **2**	20	99.20+/-29.57	13.60+/-2.62
	200	75.00+/-23.71	13.40+/-2.58
Compound **3**	20	94.40+/-37.85	17.60+/-2.29
	200	71.80+/-19.20	16.60+/-1.50
Compound **4**	20	80.20+/-20.23	14.00+/-2.07
	200	60.00+/-12.40*	18.20+/-3.32
Melipramin	25	64.30+/-14.10*	7.60+/-1.71*

Note: *significant differences in relation to the control ($p<0.05$).

It was injected intragastrically in the dose of 300 mg/kg. The control group received intragastrically the same volume of purified water. The aqueous solution of pentylenetetrazol (Sigma) in the dose of 80 mg/kg was injected subcutaneously. The anticonvulsant activity was assessed by the following indicators: the latent period of clonic or tonic convulsions, the number of clonic and tonic paroxysms per 1 mouse and mortality. The results obtained are given in Tab. 2.

The hypnotic activity of compounds **5-8** was assessed using the model of Barbamyl anesthesia in mice [2]. The test substances were injected intragastrically as water suspensions in the preventive mode in the doses of 20 and 200 mg/kg 30 min prior to the experiment. Animals of the comparison group received intragastrically the aqueous solution of Donormyl (doxylamine hydrochloride) in the dose of 20 mg/kg. The control group received intragastrically the same volume of purified water. Barbamyl in the dose of 50 mg/kg was injected intraperitoneally. The hypnotic activity was estimated by the following indicators: the latent period of anesthesia beginning ("falling asleep"), duration of the anesthesia sleep and the number of mice that were anesthetized. The results obtained are given in Tab. 3.

Results and Discussion

When studying the antidepressant activity only *N*-(1,1-diphenyl-1-hydroxyet-2-yl)-*N'*-diphenylhydroxyacetylanthranilamide (compound **4**) in the dose of 200 mg/kg reliably decreased the general duration of immobile fixation of animals in 1.9 times compared to the control group (at the same level as Imipramine). However, it had no effect on the number of immobility acts. The results ob-

Table 2

The effect of the test substances on pentylenetetrazol convulsions in mice

Group of animals / test compound	Dose, mg/kg	The latent period, min	Number of clonic and tonic paroxysms per 1 mouse	Mortality, %
Control	–	6.34+/-0.79	2.86+/-0.51	71
Compound **1**	20	6.43+/-0.71	2.60+/-0.81	40
	200	11.05+/-1.97*	1.40+/-0.25*	20**
Compound **2**	20	6.12+/-1.03	2.80+/-0.49	60
	200	4.14+/-0.44	3.00+/-0.55	80
Compound **3**	20	4.40+/-1.03	2.40+/-0.75	80
	200	5.56+/-1.41	2.00+/-0.32	80
Compound **4**	20	5.45+/-0.64	2.20+/-0.58	60
	200	5.55+/-0.95	2.60+/-0.40	60
Depakine	300	12.16+/-1.88*	1.20+/-0.41*	17**

Note: significant differences in relation to the control ($p<0.05$): * – by the Student's t-criterion; ** – by the Fisher angular transformation.

Table 3

The effect of the test substances on the Barbamyl anesthesia in mice

Group of animals / test compound	Dose, mg/kg	The latent period, min	Duration of anesthesia, min	% of mice that were anesthetized
Control	–	21.56+/-2.38	45.50+/-4.44	100
Compound **5**	20	–	0.00+/-0.00***	0##
	200	32.20	11.25*	20##
Compound **6**	20	18.53+/-1.69	15.95+/-3.12***	100
	200	9.47+/-0.48**	15.40+/-6.00**	100
Compound **7**	20	14.46+/-3.46	25.02+/-6.36*	100
	200	12.00+/-2.72*	34.11+/-6.08	100
Compound **8**	20	13.68+/-2.02*	45.82+/-7.54	100
	200	12.61+/-2.80*	43.60+/-6.75	100
Donormyl	20	11.72+/-1.46*	50.36+/-5.27	100

Note: significant differences in relation to the control: * – by the Student's t-criterion ($p<0.05$); ** – by the Student's t-criterion ($p<0.01$); *** – by the Student's t-criterion; # – by the Fisher angular transformation ($p<0.05$); ## – by the Fisher angular transformation ($p<0.01$).

tained confirm significant and dose-dependent antidepressant properties of this compound; they are slightly inferior the reference drug Imipramine.

Compounds **1-3** in both doses (except for compound **1** in the dose of 200 mg/kg) revealed a tendency to decrease the total duration of immobile fixation and the number of immobility acts. However, this difference did not reach the level of statistical significance because of high depression of data. The high activity of compound **4** can be associated with the presence of two fragments of benzylic (diphenylhydroxyacetic) acid in the structure of a molecule.

The results of the anticonvulsant activity study presented in Tab. 2 show that *N*-(phenylhydrazidoacetyl)-*N'*-succinamidoanthranilamide (compound **1**) in the dose of 200 mg/kg revealed significant anticonvulsant properties: against the background of its administration there was a statistically significant lengthening of the latent period of the clonic and tonic convulsions in 1.7 times, decrease in the number of paroxysms per 1 mouse more than twice, and also reduction of the animals' mortality by 51% ($p<0.05$) compared to the control group. The anticonvulsant effect of this compound is dose-dependent: in the dose of 20 mg/kg compound **1** does not affect the latent period and the number of convulsions per 1 mouse, and it only insignificantly reduces the mortality index in the group by 31% compared to the control group. In general, the anticonvulsant effect of this compound in the dose of 200 mg/kg is not inferior the classical anticonvulsant drug Depakine in the dose of 300 mg/kg. It caused a significant prolongation of the latent period of the first paroxysm development in the group in 1.9 times, as well as decrease in the number of convulsions in 2.4 times and the mortality index (54%) compared to the control group.

As can be seen from Tab. 3, a well-proven hypnotic drug Donormyl (doxylamine hydrochloride) in the dose of 20 mg/kg statistically significantly reduced the latent period of animals' anesthesia beginning in 1.8 times compared to the control group; however, it had no effect on the sleep duration.

Compound **5** revealed the antagonism to Barbamyl: in the dose of 20 mg/kg none of the animals were anes-

thetized, behavioural indicators were in the normal range, visual signs of retardation and depression of the CNS were not found. In the group of animals received the water solution of compound **5** in the dose of 200 mg/kg the development of classic drug-induced sleep was observed only in one animal. The results obtained indicate the stimulating effect of compound **5** on the CNS, and it requires the additional study.

Compounds **6** and **7** in the dose of 20 mg/kg had no significant effect on the latent period of animals' anesthesia beginning. However, they decreased duration of anesthesia, and it could be the evidence of the sleep structure disorder. Against the background of administration of compounds **6** and **7** in the dose of 200 mg/kg there was a significant reduction of the latent period of anesthesia beginning in 2.3 and 1.8 times compared to the control group. At the same time compound **6** showed statistically significant decrease of the sleep duration in 2.6 times.

Compound **8** in both doses reliably decreased the latent period of "falling asleep" of animals in 1.6 and 1.7 times, respectively, at the same level as the reference drug. However, it has no effect on duration of anesthesia and remained at the level of the similar value in the control group.

Therefore, it is methyl-(2-methylcarbonyloxymethyl-4-oxo-3,4-dihydro-3-quinazolinyl)acetate (compound **8**) that is a promising compound for further research of the hypnotic activity since it substantially decreases the time of "falling asleep" and does not affect duration of sleep. The analysis of the "structure-activity" relationship gives the possibility to assume that such pronounced pharmacological activity is due to the presence of substituents in position 2 of the quinazoline nucleus.

CONCLUSIONS

1. For N-(1,1-diphenyl-1-hydroxyet-2-yl)-N'-diphenylhydroxyacetylanthranilamide (compound **4**) the highest level of the antidepressant activity has been determined, it is slightly inferior than that for the reference drug Imipramine.

2. N-(phenylhydrazidoacetyl)-N'-succinamidoanthranilamide (compound **1**) reveals high anticonvulsant properties and is not inferior the action of the classical anticonvulsant drug Depakine.

3. It has been found that 2-(4-oxo-3,4-dihydro-3-quinazolinyl)acetohydrazide (compound **5**) shows the antagonistic effect in relation to Barbamyl.

4. Methyl-(2-methylcarbonyloxymethyl-4-oxo-3,4-dihydro-3-quinazolinyl)acetate (compound **8**) decreases the latent period of "falling asleep" for animals, and therefore, it is a promising biologically active substance for further research of the hypnotic activity.

REFERENCES

1. *Головенко М.Я., Громов Л.О. Доклінічне вивчення специфічної активності потенційних протисудомних препаратів: Метод. рекоменд. – К.: Авіценна, 2003. – 26 с.*
2. *Миронов А.Н., Кукес В.Г. Руководство по проведению доклинических исследований лекарственных средств. Ч. I. – М.: Гриф и К, 2012. – 244 с.*
3. *Manoj K., Srivastava S., Bharati M., Nizamuddin N. // Ind. J. Chem. – 2001. – Vol. 40. – P. 342-344.*
4. *Perumal P., Bilal A.R., Dontireddy R.S.R., Natesh R.K. // Eur. J. of Med. Chem. – 2009. – Vol. 44. – P. 2328-2333.*
5. *Porsolt R.D., Lenegre A., Elliot J.M. et al. // Experimental Approaches to Anxiety and Depression. – Chichester New York, 1992. – P. 73-85.*
6. *Sarvesh K.P., Abhishek S., Ashutosh S. // Eur. J. Med. Chem. – 2009. – Vol. 44. – P. 1188-1197.*
7. *Shemchuk L.A., Al-Asri Jamil M., Levashov D.V. et al. // Вісник фармації. – 2011. – №3. – С. 30-32.*
8. *Shemchuk L.A., Levashov D.V., Al-Asri Jamil M. et al. // ЖОФХ. – 2011. – Т. 8, №9. – С. 36-39.*
9. *Shisokoo J., Shirsath S., Rathod J.S., Yande O. // Eur. J. Med. Chem. – 2000. – Vol. 35, №3. – P. 351-358.*
10. *Usifoh C.O., Scriba K.E. // Med. Chem. – 2000. – Vol. 333. – P. 261-266.*

ФАРМАКОЛОГІЧНА АКТИВНІСТЬ ПОХІДНИХ 3,4-ДИГІДРО-4-ОКСОХІНАЗОЛІНУ ТА АНТРАНІЛАМІДІВ, ЩО МІСТЯТЬ ЗАЛИШОК ГЛІЦИНУ

Ю.О.Овсяникова, Д.В.Левашов, В.М.Кравченко, В.П.Черних, Л.А.Шемчук

***Ключові слова**: хіназолон; антраніламід; антидепресивний ефект; протисудомна активність; снодійна активність*

Похідні 3,4-дигідро-4-оксохіназоліну відомі як перспективний клас хімічних сполук, яким притаманний широкий спектр фармакологічної активності. Враховуючи дані PASS-прогнозу для одержаних нами заміщених антраніламідів та похідних 4-оксо-3,4-дигідрохіназолінів, що містять «вбудований» залишок амінокислоти гліцину в якості фармакофору, виникла підстава для дослідження центральних нейротропних ефектів. При вивченні снодійної, протисудомної та антидепресивної активності встановлені високі антидепресивні властивості N-(1,1-дифеніл-1-гідроксіет-2-іл)-N'-дифенілгідроксіацетилантраніламіду (сполука 4), який дещо поступається препарату порівняння іміпраміну. N-(фенілгідразидоацетил)-N'-сукцинамідоантраніламід (сполука 1) виявляє високі протисудомні властивості і не поступається дії класичного протисудомного засобу депакіну. При вивченні снодійного ефекту встановлено, що 2-(4-оксо-3,4-дигідро-3-хіназолініл)ацетогідразид (сполука 5) виявляє антагоністичний вплив відносно барбамілу. Метил-(2-метилкарбонілоксиметил-4-оксо-3,4-дигідро-3-хіназолініл)ацетат

(сполука 8) в дозах 20 та 200 мг/кг достовірно на рівні препарату порівняння зменшував латентний період «засинання» тварин у 1,6 та 1,7 рази відповідно і є перспективною БАР для подальших досліджень снодійної активності. Аналіз зв'язку «структура-дія» дає можливість припустити, що виразний прояв фармакологічної активності обумовлений наявністю замісників у положенні 2 хіназолінового ядра. Отримані дані дозволяють зробити висновок, що дослідження зазначених похідних є перспективним для подальшого пошуку нових біологічно активних речовин зі снодійною, протисудомною та антидепресивною властивостями.

ФАРМАКОЛОГИЧЕСКАЯ АКТИВНОСТЬ ПРОИЗВОДНЫХ 3,4-ДИГИДРО-4-ОКСОХИНАЗОЛИНА И АНТРАНИЛАМИДОВ, КОТОРЫЕ СОДЕРЖАТ ОСТАТОК ГЛИЦИНА

Ю.А.Овсяникова, Д.В.Левашов, В.Н.Кравченко, В.П.Черных, Л.А.Шемчук

***Ключевые слова**: хиназолон; антраниламид; антидепрессивный эффект; противосудорожная активность; снотворная активность*

Производные 3,4-дигидро-4-оксохиназолина известны как перспективный класс химических соединений, проявляющих широкий спектр фармакологической активности. С учетом данных PASS-прогноза для полученных нами замещенных антраниламидов и производных 4-оксо-3,4-дигидрохиназолинов, которые содержат «встроенный» остаток аминокислоты глицина в качестве фармакофора, возникли основания для исследования центральных нейротропных эффектов. При изучении снотворной, противосудорожной и антидепрессивной активности установлены высокие антидепрессивные свойства N-(1,1-дифенил-1-гидроксиэт-2-ил)-N'-дифенилгидроксиацетилантраниламида (соединение 4), который несколько уступает препарату сравнения имипрамину. N-(фенилгидразидоацетил)-N'-сукцинамидоантраниламид (соединение 1) проявляет высокие противосудорожные свойства и не уступает классическому противосудорожному средству депакину. При изучении снотворного эффекта установлено, что 2-(4-оксо-3,4-дигидро-3-хиназолинил)ацетогидразид (соединение 5) проявляет антагонистическое влияние относительно барбамила. Метил-(2-метилкарбонилоксиметил-4-оксо-3,4-дигидро-3-хиназолинил)ацетат (соединение 8) в дозах 20 и 200 мг/кг достоверно на уровне препарата сравнения уменьшает латентный период «засыпания» животных в 1,6 и 1,7 раза соответственно и является перспективным БАВ для дальнейших исследований снотворной активности. Анализ связи «структура-действие» дает возможность предположить, что выраженное проявление фармакологической активности обусловлено наличием заместителей в положении 2 хиназолинового ядра. Полученные данные позволяют сделать вывод, что исследования данных производных являются перспективными для дальнейшего поиска новых биологически активных веществ со снотворными, противосудорожными и антидепрессивными свойствами.

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

UDC 615.214:616.831-005.4+547.8

SCREENING OF DERIVATIVES OF 2-(BENZOYILAMINO)(1-R-2-OXOINDOLIN-3-YLIDENE)ACETIC ACID UNDER THE CONDITIONS OF ACUTE HYPOBARIC HYPOXIA

I.I.Zamorskii, Yu.S.Bukataru, E.L.Lenga, S.V.Kolisnyk, O.O.Altukhov

Higher State Educational Institution of Ukraine "Bukovinian State Medical University"
National University of Pharmacy

Key words: antihypoxants; hypobaric hypoxia; derivatives of 2-(benzoyilamino)(1-R-2-oxoindolin-3-ylidene)acetic acid; mexidol

*The results of screening of 2-(benzoyilamino)(1-R-2-oxoindolin-3-ylidene)acetic acid derivatives on the antihypoxic activity are presented in the article. It has been determined that under the conditions of acute hypobaric hypoxia compounds **4**, **14** and **15** have shown the increase of the integral index of the antihypoxic activity of substances – the overall lifetime of animals at the "high-altitude plateau". However, the mortality rate of animals reached 20% for compound **4**, and it significantly exceeded the control data. At the same time, compound **14** by its antihypoxic activity significantly increased the overall lifetime of animals by 150% compared to the control data, but its effect was significantly weaker than the effect of the reference drug mexidol, which increased the lifetime of animals by 197% ($p<0.05$). For compound **15** the overall lifetime of animals increased by 186% compared to the control data ($p<0.05$) and did not differ significantly from that of the reference drug. The data obtained indicate that most of the substances studied – derivatives of 2-(benzoyilamino)(1-R-2-oxoindolin-3-ylidene)acetic acid – demonstrate certain antihypoxic properties, as well as derivatives of 2-(2-oxoindolin-3-ylidene)acetic acid previously researched. Moreover, only compound **15** corresponds to the antihypoxic efficacy of the reference drug, and by the index of recovery of the animals' physical activity after their staying at the "high-altitude plateau" (the posture recovery time) it exceeds the effect of the antihypoxant drug mexidol.*

Hypoxia is a pathological condition that occurs when there is an insufficient supply of oxygen to tissues or disorder of oxygen uptake during the process of oxidation. It occurs under the conditions of oxygen deficiency in the environment, and as a result of various pathological processes and diseases associated with disorders of the respiratory and cardiovascular systems, the blood transport function or metabolism [1, 4]. In addition, very high "hypoxic risk" is related to certain professions, such as pilots, astronauts, mountaineers, alpine tourists, divers and submariners, i.e. such working conditions that are associated with the low partial pressure of oxygen in the inhaled air. In everyday life people are influenced by the physiological hypoxia. Under the physiological conditions hypoxia develops during an intense muscular work, mental activity, significantly enhanced physiological activity of the liver, kidneys and gastrointestinal tract, fetal development and in old age. Consequently, practical medicine regularly faces the problem of protecting the body from complications arising from oxygen deficiency [9]. In this regard, drugs affecting the metabolism during hypoxia – antihypoxants, which are agents that improve oxygen consumption by the body and reduce oxygen demand of tissues and organs, thereby increasing the body's resistance to oxygen deficiency, are of particular interest. A wide choice of medicines with the antihypoxic activity is presented at the pharmaceutical market of Ukraine; mexidol is considered to be one of the most active and widely used drugs [3, 7], however, in many cases its action is not sufficiently effective. Thus, the search and introduction of new effective antihypoxants into clinical practice is a topical issue of medicine and pharmacy.

The aim of the current study was to conduct screening of the antihypoxic activity among 2-(benzoyilamino)(1-R-2-oxoindolin-3-ylidene)acetic acid derivatives under the conditions of acute hypobaric hypoxia.

Materials and Methods

24 Biologically active substances (BAS) – derivatives of 2-(benzoyilamino)(1-R-2-oxoindolin-3-ylidene)acetic acid – synthesized at the Department of Analytical Chemistry of the National University of Pharmacy by professor S.V.Kolisnyk were selected for study (Fig.).

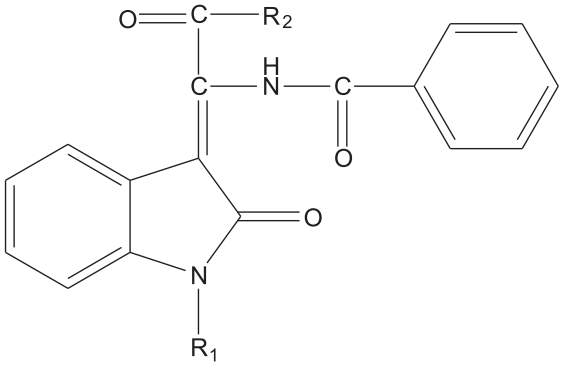


Fig. The structural formula of 2-(benzoyilamino)(1-R-2-oxoindolin-3-ylidene)acetic acid derivatives (compounds **1-24**).

Table 1

The life parameters of rats with acute hypobaric hypoxia of the critical level and when introducing 2-(benzoyilamino)(1-R-2-oxoindolin-3-ylidene)acetic acid derivatives and mexidol (M±m, n=6)

Group	Time of the posture loss, s	The lifetime till the second agonal inspiration, s	Time of the posture recovery, s
Control	64.9±9.6	15.3±8.3	398.8±9.2
Mexidol	132.8±5.8*	25.6±8.6*	225.3±9.4*
Compound **1**	66.7±4.7	25.0±3.2*	244.7±5.8*
Compound **2**	43.8±10.7	15.0±2.5	654.0±7.4*/**
Compound **3**	31.5±11.4	19.7±11.8	295.0±13.4*
Compound **4**	131.3±18.7*	27.5±12.5	363.2±14.4
Compound **5**	60.0±10.6	14.0±5.2	240.0±10.9*
Compound **6**	57.5±7.8	27.5±5.2	263.6±6.9*
Compound **7**	19.4±10.4*	9.6±7.2	333.0±10.0
Compound **8**	23.0±5.9	9.2±8.3	257.3±4.2*
Compound **9**	42.5±7.8	51.3±3.9*/**	278.2±6.2*
Compound **10**	23.0±10.5	28.0±9.8	264.2±10.0*
Compound **11**	27.5±4.3	12.8±5.8	215.4±6.3*
Compound **12**	39.0±10.3	29.2±9.2	310.8±7.5*
Compound **13**	23.0±9.5	14.0±10.3	205.2±8.8*
Compound **14**	69.5±8.2	51.4±7.6*/**	265.4±5.7*
Compound **15**	126.0±10.5*	23.5±5.7*	146.3±8.5*/**
Compound **16**	13.4±8.4*	17.4±9.3	262.5±7.4*
Compound **17**	15.5±5.4*	9.8±3.8	312.8±4.2*
Compound **18**	61.0±6.7	37.8±4.8*	241.0±10.6*
Compound **19**	52.2±9.3	24.4±7.3	308.0±10.7*
Compound **20**	25.2±4.8	19.0±5.2	276.2±9.4*
Compound **21**	53.8±7.4	17.4±6.4	289.8±9.7*
Compound **22**	19.8±3.9*	3.0±0.5*	345.8±9.4
Compound **23**	65.0±7.4	42.0±4.6*	341.4±8.7
Compound **24**	70.0±6.5	28.0±7.5	309.8±7.3*

Note: * – significance compared to the control ($p<0.05$); ** – significance compared to mexidol ($p<0.05$).

2-(Benzoyilamino)(1-R-2-oxoindolin-3-ylidene) acetic acids (compounds **1-4**), and their phenyl- (compounds **5-11**), naphthalen- (compounds **12-14**) phenethyl- (compounds **15-17**), hydroxynaphthalenamides (compounds **23-24**) and ethyl esters of N-[2-(benzoyilamino) (2-oxoindolin-3-ylidene)acetyl]glycine (compounds **18-22**) were studied.

The animals were kept under the standard vivarium conditions at a constant temperature and humidity with free access to food and water. All manipulations were carried out in accordance with the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes.

The research was conducted under the conditions of acute hypoxia on 156 nonlinear white mature male rats weighing 180-200 g aged 3 months and moderately resistant to hypoxia. The resistance of animals to hypoxia was determined 2 weeks prior to the research by the known method [2]. Acute hypobaric hypoxia was simulated in the modified flow pressure chamber by imitation of the lifting of rats to an altitude of 12000 metres. "Ascent" and "descent" of animals were carried out at a speed of 50 m/s. At the "high-altitude plateau" rats were maintained until the second agonal inspiration, and then the "descent" to the previous zero altitude was performed [11]. The substances studied were administered intraperitoneally 35 min before hypoxia modelling in the dose of 15 mg/kg in the form of an aqueous suspension stabilized by polysorbate 80 (Tween 80) (AppliChem GmbH, Germany) [6, 8]. The reference drug – antihypoxant mexidol (ethylmethylhydroxypyridine succinate) was administered in the dose of 100 mg/kg [10]. The animals of the control group were injected with an equivalent amount of an aqueous suspension with polysorbate 80. Doses of substances were selected according to the published data concerning the antihypoxic activity in experimental studies.

The antihypoxic activity of substances was assessed by the animals' survival indices at the "high-altitude plateau": the time of the posture loss; the lifetime – the time till the second agonal inspiration; the posture recovery time after termination of hypoxia and a gradual return of animals to the previous zero altitude; and the overall lifetime of animals – summation of the time of the posture loss and the lifetime [5].

Statistical analysis of the results was performed using SPSS Statistics 17.0 and Microsoft Excel 2013 software. Statistical significance was assessed using parametric Student's t-test (for normal distribution) and non-parametric Mann-Whitney U-test (in case of non-normal distribution). The critical level of significance was accepted as $p \leq 0.05$.

Results and Discussion

The results of the antihypoxic activity screening of the compounds studied compared to the control group and the action of the reference drug mexidol are presented in Tab. 1.

Analysis of screening 2-(benzoyilamino)(1-R-2-oxoindolin-3-ylidene)acetic acid derivatives have shown that the most significant prolongation of life parameters in acute hypobaric hypoxia of the critical level according to the highest index of the lifetime at the "high-altitude plateau" after the loss of posture till the second agonal inspiration ($p<0.05$) have animals treated with compounds **1**, **4**, **9**, **14**, **15**, **18**, **23** and **24** (Tab. 1). The highest index of the time of the posture loss at the "high-altitude plateau" was for 2-(benzoyilamino)(1-propyl-2-oxoindolin-3-ylidene)acetic acid (compound **4**) and for phenylethylamide 2-(benzoyilamino)(1-methyl-2-oxoindolin-3-ylidene)acetic acid (compound **15**), it exceeded the control data by 2 and 1.9 times ($p<0.05$), respectively. The index of the posture recovery time after the beginning of the "descent" of animals from the "high-altitude plateau" was significantly lower compared to the control data for almost all BAS under research except compounds **2**, **4**, **7**, **22**, **23**. In the group of phenylethylamide **15** this index was the lowest, and it significantly exceeded the antihypoxic efficacy of the reference drug mexidol by 1.5 times.

After administration of compounds **4**, **9**, **18**, **23** and **24** such adverse reactions as convulsions and cyanosis of the skin and mucous membranes were observed. It was also found that among substances exhibiting the significant antihypoxic activity only compounds **14** and **15** did not cause any external signs of side effects after their administration when modelling hypoxia.

Compounds **4**, **14** and **15** demonstrated the increase of the integral index of the antihypoxic activity of substances – the overall lifetime of animals at the "high-altitude plateau" (Tab. 2). However, the mortality rate of animals reached 20% for compound **4**, and it significantly exceeded the control data. At the same time, amide **14** by its antihypoxic activity significantly increased the overall lifetime of animals by 150% compared to the control data, but its effect was significantly weaker than the effect of the reference drug mexidol, which increased the lifetime of animals by 197% ($p \leq 0.05$). For compound **15** the overall lifetime of animals increased by 186% compared to the control data ($p \leq 0.05$) and did not differ significantly from that of the reference drug.

Table 2

The integral antihypoxic activity of some 2-(benzoyilamino)(1-R-2-oxoindolin-3-ylidene) acetic acid derivatives under the conditions of acute hypobaric hypoxia compared to the action of mexidol (M±m; n=6)

Group	Overall lifetime, s	Activity in relation to the control, %	Activity in relation to mexidol, %
Control	80.2±17.9		
Mexidol	158.4±14.4*	197	100
Compound **4**	158.8±31.2*	198	100
Compound **14**	120.9±15.8*/**	150	76
Compound **15**	149.5±16.2*	186	94

Note: * – significance compared to the control ($p<0.05$);
** – significance compared to mexidol ($p<0.05$).

The data obtained indicate that most of the substances studied – derivatives of 2-(benzoyilamino)(1-R-2-oxoindolin-3-ylidene)acetic acid – demonstrate certain antihypoxic properties, as well as derivatives of 2-(2-oxoindolin-3-ylidene)acetic acid previously researched [8]. Moreover, only compound **15** corresponds to the antihypoxic efficacy of the reference drug, and by the index of recovery of the animals' physical activity after their staying at the "high-altitude plateau" (the posture recovery time) it exceeds the effect of the antihypoxant drug mexidol.

CONCLUSIONS

1. The results of screening have shown that derivatives of 2-(benzoyilamino)(1-R-2-oxoindolin-3-ylidene) acetic acid are a promising class of compounds for creating antihypoxic medicines on their basis, and it is the basis for further pre-clinical studies of pharmacological properties of these compounds.

2. Compound **15** corresponds to the action of the reference drug mexidol by its antihypoxic activity, and significantly exceeds its effect by the index of recovery of the physical activity after the animals' staying at the "high-altitude plateau".

REFERENCES

1. *Андреева Н.Н. // Мед. альманах. – 2009. – №4 (9). – С. 193-197.*
2. *Березовский В.А. Гипоксия и индивидуальные особенности реактивности. – К.: Наук. думка, 1978. – 216 с.*
3. *Воронина Т.А. // Журн. неврол. и психиатр. им. С.С.Корсакова. – 2012. – №12. – С. 86-90.*
4. *Евсеева М.А., Евсеев А.В., Правдивцев В.А. и др. // Обзоры по клин. фармакол. и лек. терапии. – 2008. – Т. 6, №1. – С. 3-25.*
5. *Заморський І.І. // Одеський мед. журн. – 1998. – №6 (50). – С. 23-25.*
6. *Зыкова С.С. // Вестник современ. клин. мед. – 2014. – Т. 7, вып. 2. – С. 70-73.*

7. *Катунина Н.П. // Наука и современность. – 2010. – №5. – С. 298-300.*
8. *Колісник С.В., Кононенко Н.М., Гаман Д.В. та ін. // Вісник фармації. – 2011. – №4 (68). – С. 64-66.*
9. *Лукьянова Л.Д. // Физиол. журн. – 2003. – Т. 49, №3. – С. 17-35.*
10. *Sevryukov O.V., Volkovoy V.A., Kolisnyk O.V. et al. // Вісник фармації. – 2015. – №3 (83). – С. 76-78.*
11. *Zamorskii I.I., Sopova I.Yu., Khavinson V.Kh. // Bull. Exp. Biol. Med. – 2012. – Vol. 154, №1. – P. 51-53.*

СКРИНІНГ ПОХІДНИХ 2-(БЕНЗОЇЛАМІНО)(1-R-2-ОКСОІНДОЛІН-3-ІЛІДЕН)ОЦТОВОЇ КИСЛОТИ ПРИ ГОСТРІЙ ГІПОБАРИЧНІЙ ГІПОКСІЇ

І.І.Заморський, Ю.С.Букатару, Е.Л.Ленга, С.В.Колісник, О.О.Алтухов

***Ключові слова**: антигіпоксанти; гіпобарична гіпоксія; похідні 2-(бензоїламіно)(1-R-2-оксоіндолін-3-іліден)оцтової кислоти; мексидол*

*Наведені результати скринінгу похідних 2-(бензоїламіно)(1-R-2-оксоіндолін-3-іліден)оцтової кислоти на антигіпоксантну активність. Встановлено, що за умов гострої гіпобаричної гіпоксії збільшення інтегрального показника антигіпоксантної активності речовин – загальний час життя тварин на «висотному плато» – демонстрували речовини під номерами **4**, **14** і **15**. Проте для речовини **4** рівень смертності тварин при проведенні досліджень становив 20%, що значно перевищувало контрольні дані. При цьому сполука під номером **14** за антигіпоксантною активністю достовірно збільшувала загальну тривалість життя тварин на 150% щодо даних контролю, але вірогідно поступалась препарату порівняння мексидолу: цей лікарський засіб збільшував час життя тварин на 197% ($p<0,05$). Для речовини під номером **15** загальна тривалість життя тварин зростала на 186% у порівнянні з даними контролю ($p<0,05$) і вірогідно не відрізнялася від показників референс-препарату. Отримані дані вказують на те, що більшість досліджуваних речовин, похідних 2-(бензоїламіно)(1-R-2-оксоіндолін-3-іліден)оцтової кислоти проявляють певні антигіпоксантні властивості. Водночас тільки речовина за номером **15** не поступається за антигіпоксантною ефективністю дії препарату порівняння, а за показником відновлення фізичної активності тварин після їх перебування на «висотному плато» (часом відновлення пози) перевершує дію відомого антигіпоксанта мексидолу.*

СКРИНИНГ ПРОИЗВОДНЫХ 2-(БЕНЗОИЛАМИНО)(1-R-2-ОКСОИНДОЛИН-3-ИЛИДЕН) УКСУСНОЙ КИСЛОТЫ ПРИ ОСТРОЙ ГИПОБАРИЧЕСКОЙ ГИПОКСИИ

И.И.Заморский, Ю.С.Букатару, Э.Л.Ленга, С.В.Колесник, А.А.Алтухов

***Ключевые слова**: антигипоксанты; гипобарическая гипоксия; производные 2-(бензоиламино)(1-R-2-оксоиндолин-3-илиден)уксусной кислоты; мексидол*

*Приведены результаты скрининга производных 2-(бензоиламино)(1-R-2-оксоиндолин-3-илиден) уксусной кислоты на антигипоксантную активность. Установлено, что в условиях острой гипобарической гипоксии увеличение интегрального показателя антигипоксантной активности веществ – общее время жизни животных на «высотном плато» – демонстрировали вещества под номерами **4**, **14** и **15**. Однако, для вещества **4** уровень смертности животных при проведении исследований составил 20%, что значительно превышало контрольные данные. При этом соединение под номером **14** по антигипоксантной активности достоверно увеличивало общую продолжительность жизни животных на 150% относительно данных контроля, но достоверно уступало препарату сравнения мексидолу: это лекарственное средство увеличивало время жизни животных на 197% ($p<0,05$). Для вещества под номером **15** общая продолжительность жизни животных возрастала на 186% по сравнению с данными контроля ($p<0,05$) и достоверно не отличалась от показателей референс-препарата. Полученные данные указывают на то, что большинство исследуемых веществ, производных 2-(бензоиламино)(1-R-2-оксоиндолин-3-илиден)уксусной кислоты проявляют определенные антигипоксантные свойства. В то же время только вещество под номером **15** не уступает по антигипоксантной эффективности действию препарата сравнения, а по показателю восстановления физической активности животных после их пребывания на «высотном плато» (время восстановления позы) превосходит действие известного антигипоксанта мексидола.*

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

UDC 615.272/451.16::582.893:[615.214+612.744.24]

THE EFFECT OF MEDICINES WITH GOUTWEED (*AEGOPODIUM PODAGRARIA L.*) ON THE PHYSICAL ENDURANCE, COGNITIVE FUNCTIONS AND THE LEVEL OF DEPRESSION IN ANIMALS

O.V.Tovchiga, S.Yu.Shtrygol'

National University of Pharmacy

Key words: goutweed (Aegopodium podagraria L.); extract; tincture; central nervous system; physical endurance

The effect of the extract (100 mg/kg and 1 g/kg) and the tincture (1 and 5 ml/kg) of the goutweed (Aegopodium podagraria L.) aerial part on the physical endurance, the level of depression and cognitive functions has been studied using the weight-loaded forced swimming test, the extrapolation escape test, and the reserpine-induced depression model. The goutweed extract in the dose of 100 mg/kg, but not in the dose of 1 g/kg, significantly increases the exhaustive swimming time (10% and 20% load) in male mice. In female mice the augmentation of exhaustive swimming time is registered with 20% load against the background of the extract in both doses. The goutweed tincture does not change the results of this test. Goutweed medicines have the ambiguous effect on the results of the extrapolation escape test: the extract and the tincture do not change the percentage of mice that succeed in completing the task, still the average time spent for the task performance is significantly decreased in these animals (but not within the whole group) against the background of the extract in the doses of 100 mg/kg and 1 g/kg. The extract in the dose of 100 mg/kg significantly reduces the number of rats capable of completing the task, the extract (1 g/kg) and the tincture (1 ml/kg) increase the time spent for performing the extrapolation escape task by rats. The extract and the tincture do not change the body temperature reduction and blepharoptosis induced by reserpine in rats. Thus, the goutweed extract is able to increase the physical endurance, exert a moderate positive effect on cognitive functions in mice (but not in rats) without any significant changes in the level of depression. The goutweed tincture worsens the results of the extrapolation escape test in the dose of 1 ml/kg, does not change them in the dose of 5 ml/kg and does not influence on the level of depression and physical endurance in both doses.

According to the WHO forecast, in 2020 depression will become the second leading cause of disability, therefore, considerable efforts are directed towards the improvement of the methods of its treatment [15], including the study of the promising herbal drugs. Besides antidepressants of the plant origin traditionally used (such as *Hypericum perforatum L., Clusiaceae*), herbal drugs (HD) combining a favourable metabolic activity and a mild psychotropic effect have arisen a great interest. On the one hand, the complex composition of crude HD is a prerequisite enabling useful concomitant effects, among which the psychotropic action may be supposed. On the other hand, for the HD that are used or planned to be used in chronic diseases for a long time the safety verification, including effects on the CNS, is particularly relevant.

Our research focuses on the verification of pharmacological properties of goutweed (*Aegopodium podagraria L. Apiaceae*, GW) since the drugs obtained from this plant have long been used in folk medicine for the treatment of kidney diseases and metabolic disorders [3]. The dry extract and the tincture were obtained from the GW aerial part, their favourable effect on the purine metabolism, the carbohydrate metabolism, as well as a significant nephroprotective and hepatoprotective activity of the extract and its ability to counteract the effects of ethanol were shown [2, 4, 7]. The study of the psychotropic effects of these medicines in intact mice was started [17], and the antidepressant action of the extract (in the dose of 100 mg/kg, but not in the dose of 1 g/kg in female mice) with worsening of the results of the passive avoidance response (PAR) test was registered. The tendency towards reduction of anxiety signs in animals of both sexes against the background of the extract in the dose of 100 mg/kg (in males also against the background of the extract in the dose of 1 g/kg and the tincture in the doses of 1 and 5 ml/kg) was also registered. As evidenced from the foregoing, it is expedient to continue the study of the effect of GW medicines on the level of the animals' depression, as well as on the cognitive processes. Taking into consideration the favourable metabolic activity of GW medicines [2, 7] and the extract ability to decrease duration of immobility of animals [17] it is also efficient to determine the effect of these herbal medicines on the physical performance.

The aim of this study was to evaluate the efficacy of medicines with *A. podagraria L.* in the weight-loaded forced swimming test, the extrapolation escape test, as well as reserpine-induced depression.

Materials and Methods

The dry extract and the tincture were obtained from *A. podagraria L.* aerial part using the standard method described previously in accordance with the requirements of the State Pharmacopoeia of Ukraine [2, 7].

All the experiments were conducted according to the principles of bioethics as required in the "Directive 2010/63/EU of the European Parliament and of the Council of September, 22, 2010 on the protection of animals used for scientific purposes." Albino male rats with the body weight of 180-220 g and albino male mice with the body weight of 16-22 g were kept in the Central Research Laboratory of the National University of Pharmacy under standard conditions. As the gender specific effect of GW medicines on the immobility time was registered [17], female mice with the body weight of 18-24 g were also used in the weight-loaded forced swimming test.

In all the experiments discussed GW medicines, namely the extract as a water solution in the doses of 100 mg/kg and 1 g/kg and the tincture, from which ethyl alcohol was previously removed, in the doses of 1 and 5 ml/kg were administered to mice or rats intragastrically in the preventive mode. The animals of the intact control groups received the same amount of water. The last dose of these medicines (or water) in all the experiments was administered 40 min before beginning of the tests or reserpine administration.

The weight-loaded forced swimming test was conducted after the course of administration of GW medicines (10 doses). The metal load (10% of the body weight) was fixed on the tail root of each mouse, and the animals were placed individually into the pool with water at 22-23°C. The pool was filled with 60 cm water, the pool ledges equalled 15 cm over the water level without allowing the rest of the animal on them. The swimming time to exhaustion was recorded by the criterion of head dip under water without coming to the water surface for 10 s [1]. To determine the stability of the effect and its limits one day after the test was repeated with 20% load. In this test additional groups of mice were used. They received such reference drugs as the extract of St. John's wort (*Hypericum perforatum L., Clusiaceae*) ("Deprivit" from Kyiv Vitamin Factory, Ukraine) as a herbal drug being effective in the experimental pharmacology [14] with the proven ability to stimulate the CNS in the dose of 100 mg/kg intragastrically, and the extract of *Passiflora incarnata L. (Passifloraceae)* as "Alora" syrup (NOBEL ILAC Sanayii ve Ticaret A.S., Turkey) being effective according to the data [12] in the dose of 300 mg/kg intragastrically. The latter was used as a reference drug because in the previous experiments the reduction of anxiety signs in animals receiving the GW extract and the passionflower extract appeared to be similar [17]. Besides, along with the extract of *H. perforatum L.*, which mainly leads to the CNS activation [14], it is expedient to use other well studied and practically applied reference drug, which main psychotropic effects are anxiolytic and sedative [12].

The cognitive functions of mice and rats receiving GW medicines (the course lasted 7 days) were assessed by extrapolation escape test registering the latency of escape (avoidance through diving) of the animal placed to the cylinder with the edge under water as described in [11]. The test period was limited to 120 s (mice) and 180 s (rats), and the number of animals performed the task was recorded. In these experiments 5 groups of mice and 5 groups of rats were used (the control group and animals receiving GW medicines in the doses mentioned).

In the experiments in rats the efficacy of GW medicines (the course of preventive administration lasted 7 days) was also determined under the conditions of a single intraperitoneal administration of reserpine in the dose of 4 mg/kg. The depressogenic effect of reserpine was assessed in 7 h using the criteria of the decrease in the body temperature (rectal measurement) and the blepharoptosis rate (the degree of ptosis was rated according to the following rating scale: 0 – complete absence of ptosis, 1 – eyes are half closed, 2 – the eye closure is more than on ½, 3 – eyes are completely closed) [6, 9].

Proceeding from the modern requirements for medical and biological data analysis [15], medians, 25% and 75% percentiles (upper and lower quartiles) were calculated.

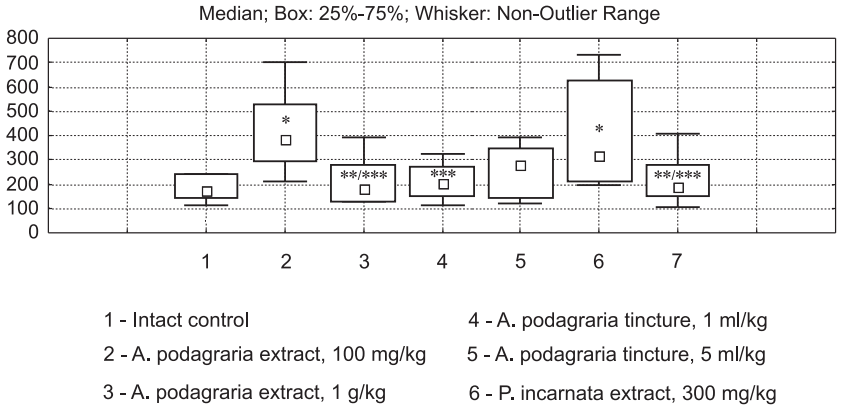


Fig. 1. The effect of medicines with *Aegopodium podagraria L.* and reference drugs on the physical endurance of male mice in the weight-loaded (10% of the body weight) forced swimming test, seconds. * – statistically significant differences compared to the intact control group, $p<0.05$; ** – statistically significant differences compared to the group receiving the extract of *Aegopodium podagraria L.* in the dose of 100 mg/kg, $p<0.02$; *** – statistically significant differences compared to the group receiving the extract of *Passiflora incarnata L.*, $p<0.05$.

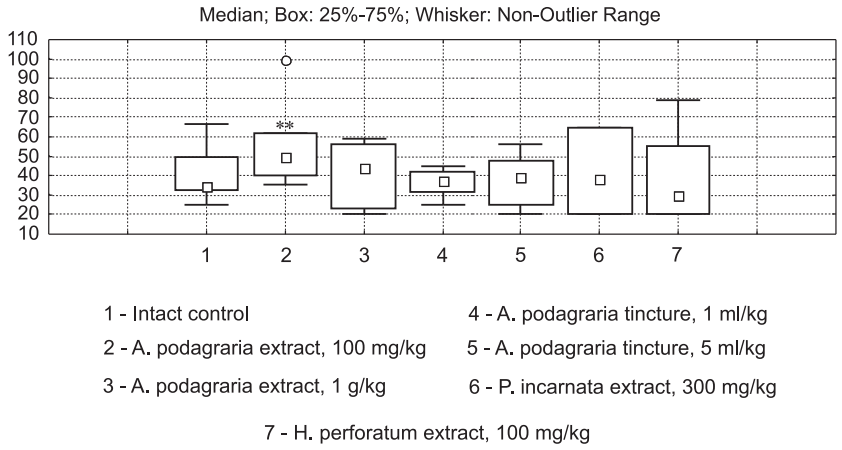


Fig. 2. The effect of medicines with *Aegopodium podagraria L.* and reference drugs on the physical endurance of male mice in the weight-loaded (20% of the body weight) forced swimming test, seconds. ** – statistically significant differences compared to the intact control group, p<0.02.

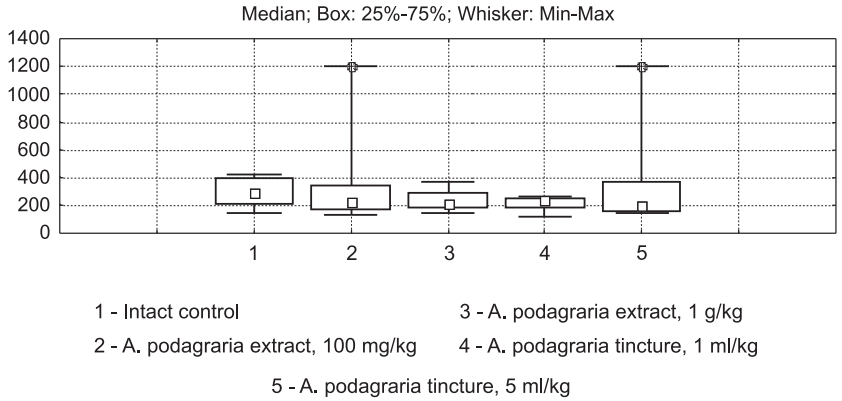


Fig. 3. The effect of medicines with *Aegopodium podagraria L.* on the physical endurance of female mice in the weight-loaded (10% of body weight) forced swimming test, seconds.

Traditionally used means ± standard errors of the mean (SEM) are also presented (M±m). Central tendencies of the independent samples were compared using the Mann-Whitney U test, intergroup differences (the body temperature before and after reserpine administration) were analysed using the Wilcoxon matched pairs T̃ test, and the Fisher's angular transformation was used for comparing data represented in alternative forms.

Results and Discussion

The weight-loaded forced swimming test is a generally accepted test for the study of physical endurance of animals [1], but its results can be changed significantly in animals receiving psychotropic drugs (such as stimulation of motor activity against the background of medicines that activate the CNS, and decrease in physical performance after administration of sedative and anxiolytic drugs [8]). As shown in Fig. 1 and Fig. 2, the GW extract in the dose of 100 mg/kg, but not in the dose of 1 g/kg, significantly increased duration of swimming to exhaustion in male mice for both regimens used. In female mice receiving the GW extract in both doses the effect was manifested only when 20% load was used (Fig. 3, Fig. 4, with 10% load only drastic augmentation of physical performance in certain animals was seen after administration of the extract in the lower dose and the tincture in the high dose). The mechanism of action of the extract (which requires further research) can be associated with favourable peripheral metabolic effects (hypoazotemic action, optimization of the glucose metabolism, cytolysis counteraction, etc., though they are more marked against the background of the extract in the high dose [2, 7]), as well as with the psychotropic activity of this medicine. The latter assumption is indirectly supported by the data about the decrease in duration of immobility and a tendency towards reduction of anxiety signs in mice receiving this herbal medicine in the dose of 100 mg/kg [17] and the presence of the extract effect during the second session of swimming in mice since it is known that the effect on the level of depression is revealed just in the repeated tests [16]. As to the reference drugs *H. perforatum L.* extract did not significantly affect duration of swimming in male mice, while *P. incarnata L.* extract increased this value (statistically significant increase in the test with 10% load and a tendency in the test with 20% load). In most studies available the effect of these well-studied herbal drugs was determined in the forced swimming test with registration of the swimming and immobilization time for a certain period, but not with the general duration of swimming. According to these data both reference drugs increased duration of swimming in the doses similar to those used in our study, namely 70 mg/kg for the extract of *H. per-*

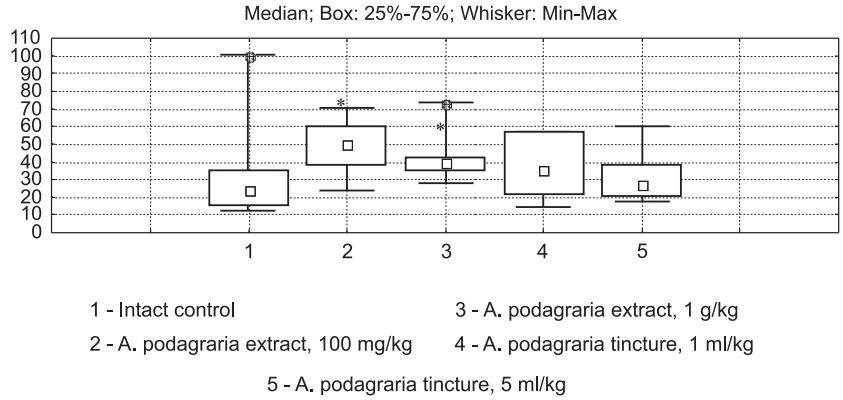


Fig. 4. The effect of of medicines with *Aegopodium podagraria L.* on the physical endurance of female mice in the weight-loaded (20% of body weight) forced swimming test, seconds. * – statistically significant differences compared to the intact control group, p<0.05.

foratum L. (but after a single administration) [10], 200 and 400 mg/kg for the extract of *P. incarnata L.* [13].

The extrapolation escape test characterizing the ability of an animal to find a way out in the acute stressful situation [11] was conducted to broaden the data about psychotropic effects of the medicines studied since previously it was shown that there was a worsening in PAR test results in female (but not in male) mice against the background of the extract in the dose of 100 mg/kg; exactly in this dose the extract decreased duration of immobility and tended to reduce the signs of anxiety [17]. As shown in Tab. 1, GW medicines did not influence on the results of this test in mice (only a tendency towards worsening of the results was observed against the background of the tincture in the lower dose); however, after administration of the extract in the dose of 100 mg/kg in rats there was a significant reduction in the number of animals that were able to escape. In addition, augmentation of the time spent for performing the task was registered in rats under the influence of the extract in a high dose and the tincture in a low dose. However, the time spent for performing the task in animals that were able to escape tended to decrease in rats receiving the extract in the dose of 100 mg/kg (with the opposite change in animals treated with this medicine in the dose of 1 g/kg) and also was significantly reduced in groups of mice receiving the extract in both doses. Thus, summarizing the experimental data of the present and previous works [17] shows that the lowering of anxiety signs and emotional reactions (grooming) in male mice receiving the GW extract is not accompanied with negative changes in the results of both PAR and extrapolation escape test.

The extrapolation escape test is widely used for the research of nootropic drugs under complicated conditions (stressful situation, trauma, effect of toxins, etc.), but there is no information in the available literature about the effect of herbal biologically active substances on the results of this test. Taking into account interspecies diffe-

Table 1

The effect of medicines with *Aegopodium podagraria L.* on the results of the extrapolation escape test in male rats and male mice, M±m; $\mathbf{Q_{50}}$ ($\mathbf{Q_{25}}$-$\mathbf{Q_{75}}$)

Group	Rats			Mice		
	Latency of escape, s		Number of animals that were able to escape, % (absolute number)	Latency of escape, s		Number of animals that were able to escape, % (absolute number)
	In the whole group	Among animals that were able to escape		In the whole group	Among animals that were able to escape	
Intact control, n=9, n=8	44±17 21 (15-26)		100 (9/9)	28±13 15 (14-18)	15±2 15 (13-16)	88 (7/8)
A. podagraria extract, 100 mg/kg, n=6, n=8	101±36 110 (21-180)	21±9 15 (12-28)	50 (3/6)*	29±15 9 (7-26)	16±8 8 (7-12)*	88 (7/8)
A. podagraria extract, 1 g/kg, n=6, n=8	99±29 89 (41-166)*	59±22 46 (33-73)	67 (4/6)	36±18 10 (6-40)	8±2 6 (6-11)**	75 (6/8)
A. podagraria tincture, 1 ml/kg, n=5, n=8	114±36 160 (27-180)*	71±45 27 (26-94)	60 (3/5)	59±20 47 (8-120)	23±16 8 (6-8)	63 (5/8)
A. podagraria tincture, 5 ml/kg, n=7, n=8	45±23 26 (16-37)	23±5 22 (15-33)	86 (6/7)	23±14 10 (5-16)	9±2 9 (5-13)	88 (7/8)

Notes: * – statistically significant differences compared to the intact control group, p<0.05; ** – statistically significant differences compared to the intact control group, p<0.01.

Table 2

The effect of medicines with *Aegopodium podagraria L.* on the body temperature decrease and blepharoptosis in reserpine-treated rats, M±m; $\mathbf{Q_{50}}$ ($\mathbf{Q_{25}}$–$\mathbf{Q_{75}}$)

Group	Body temperature, °C		Body temperature decrease, °C	Blepharoptosis, points
	basal level	4 hours after reserpine administration		
Intact control, n=8	38.9±0.08 38.9 (38.8-39.0)	38.1±0.21 38.2 (38.0-38.4)**	0.7±0.3 0.7 (0.2-1.0)	2.0±0.4 2.0 (1.8-3.0)
A. podagraria extract, 100 mg/kg, n=6	39.3±0.09 39.3 (39.1-39.5)	38.3±0.18 38.2 (38.0-38.4)*	1.0±0.2 1.1 (0.9-1.3)	1.8±0.5 2.0 (1.3-2.8)
A. podagraria extract, 1 g/kg, n=7	39.3±0.12 39.4 (39.2-39.5)	38.3±0.30 38.4 (37.8-39.1)*	1.0±0.3 1.0 (0.3-1.6)	1.4±0.4 2.0 (0.5-2.0)
A. podagraria tincture, 1 ml/kg, n=7	39.3±0.10 39.2 (39.2-39.3)	38.2±0.20 38.1 (37.9-38.6)*	1.1±0.2 1.2 (1.1-1.3)	1.2±0.4 1.0 (0.5-2.0)
A. podagraria tincture, 5 ml/kg, n=7	39.1±0.20 39.1 (39.1-39.3)	38.3±0.11 38.3 (38.1-38.5)**	0.8 ±0.1 0.8 (0.6-1.0)	0.9±0.3 1.0 (0-1.5)

Notes: * – statistically significant differences compared to the basal level, $p<0.05$; ** – statistically significant differences compared to the basal level, $p<0.02$.

rences and the possible absence of correlation between the results of different psychopharmacological tests, including PAR and extrapolation escape test (and the concept about the dependence of PAR results on the overall strategy of animal's behaviour [5], that is likely to be changed under the influence of herbal drugs eliminating anxiety and depression signs), further studies of psychotropic effects of GW medicines are expedient (especially in female animals because of more significant changes in their behavioural reactions [17]).

The test of interaction with reserpine that causes depletion of catecholamines is commonly used for elucidation of the mechanism of action of psychotropic drugs from different groups despite the absence of high selectivity of reserpine [6]. As can be seen from Tab. 2, reserpine significantly reduced the body temperature of animals and caused blepharoptosis, the results obtained were comparable with the data in the literature [9]. There were no intergroup differences in the parameters studied (only a tendency towards blepharoptosis reduction was present against the background of the GW tincture). Hence, the effect of GW biologically active substances is probably not related to catecholaminergic mechanisms. Since the GW extract can decrease duration of immobility of animals, further studies of its mechanisms of action are expedient.

CONCLUSIONS

1. In the weight-loaded forced swimming test the extract of *Aegopodium podagraria L.* in the dose of 100 mg/kg, but not in the dose of 1 g/kg, significantly increases the physical endurance in male mice (with 10% and 20% load used). In female mice this effect is registered with 20% load against the background of the extract in the doses of 100 mg/kg. The tincture of *Aego-podium podagraria L.* does not cause a favourable effect on the physical endurance.

2. The extract and tincture of *Aegopodium podagraria L.* do not change the percentage of mice that succeed in completing the extrapolation escape task; however, among these animals (but not within the whole group) the extract in the doses of 100 mg/kg and 1 g/kg significantly decreases the latency of escape, indicating moderate nootropic properties. However, the extract in the dose of 100 mg/kg, in contrast, significantly reduces the number of rats capable of completing the task; the extract in the dose of 1 g/kg and the tincture in the dose of 1 ml/kg increase the time spent for the task performance in rats.

3. The extract and tincture of *Aegopodium podagraria L.* do not influence on the decrease in the body temperature and blepharoptosis caused by reserpine in rats, and therefore, do not exhibit antidepressant properties.

REFERENCES

1. *Експериментальне вивчення нових адаптогенних засобів: Метод. рекоменд. / Л.В.Яковлєва, О.Я.Міщенко, Ю.Б.Лар'яновська та ін. – К., 2009. – 37 с.*
2. *Койро О.О.: автореф. дис. ... канд. фарм. наук. – Х., 2014. – 20 с.*
3. *Лікарські рослини: Енциклопед. довід. / Відп. ред. А.М.Гродзинський. – К.: Голов. ред. УРЕ, 1990. – С. 487.*
4. *Пат. на кор. модель 95127 Україна, МПК A 61 P 25/32 (2006.01), A 61 K 36/23(2006.01) / О.В.Товчига, С.Ю.Штриголь, В.А.Товчига; заявник та власник НФаУ. – № и 2014 07336. Заявл.: 01.07.2014. Опубл.: 10.12.2014. – Бюл. №23/2014.*
5. *Райт Д.В., Грошев Д.С. Матер. Междунар. молодежного научного форума «ЛОМОНОСОВ-2014». – М.: МАКС Пресс, 2014. – С. 7-8.*

6. *Руководство по экспериментальному (доклиническому) изучению новых фармакологических веществ / Под общ. ред. чл.-кор. РАМН, проф. Р.У.Хабриева. – М.: Мед., 2005. – С. 247.*
7. *Товчига О.В.: автореф. дис. ... канд. фарм. наук. – Х., 2009. – 21 с.*
8. *Тюренков И.Н., Меркушенкова О.В., Багметова В.В. // Вестник ВолГМУ. – 2008. – №4 (28). – С. 83-86.*
9. *Щокіна К.Г., Штриголь С.Ю., Іщенко О.М., Бухтіярова І.П. // Вісник фармації. – 2010. – №4 (64). – С. 71-74.*
10. *Bach-Rojecky L., Kalodjera Z., Samarzija I. // Acta Pharm. – 2004. – Vol. 54, №2. – P. 157-162.*
11. *Bondarenko N.A., Miroshnichenko I.I., Kudrin V.S. // Bull. Exp. Biol. Med. – 1990. – Vol. 110, №11. – P. 506-508.*
12. *Deng J., Zhou Y., Bai M. et al. // J. Ethnopharmacol. – 2010. – Vol. 128, №1. – P. 148-153.*
13. *Jafarpoor N., Abbasi-Maleki S., Asadi-Samani M., Khayatnouri M.H. // J. Herb Med. Pharmacol. – 2014. – Vol. 3, №1. – P. 41-45.*
14. *Kumar V., Singh P.N., Jaiswal A.K., Bhattacharya S.K. // Ind. J. Exp. Biol. – 1999. – Vol. 37, №12. – P. 1171-1176.*
15. *Mental health: a call for action by world health ministers / WHO background document. – Geneva: WHO, 2001. – 23 p.*
16. *Sun M.K., Alkon D.L. // J. Neurosci Meth. – 2003. – Vol. 126. – P. 35-40.*
17. *Tovchiga O., Shtrygol' S. // J. Chem. Pharm. Res. – 2015. – Vol. 7, №7. – P. 370-384.*
18. *Weissgerber T.L., Milic N.M., Winham S.J., Garovic V.D. // PLOS Biol. – 2015. – Vol. 13, №4. – Art. e1002128.*

ВПЛИВ ПРЕПАРАТІВ ЯГЛИЦІ ЗВИЧАЙНОЇ *(AEGOPODIUM PODAGRARIA L.)* НА ФІЗИЧНУ ВИТРИВАЛІСТЬ, КОГНІТИВНІ ФУНКЦІЇ ТА ДЕПРЕСИВНІСТЬ ТВАРИН

О.В.Товчига, С.Ю.Штриголь

***Ключові слова**: яглиця звичайна (Aegopodium podagraria L.); екстракт; настойка; центральна нервова система; фізична витривалість*

Досліджено вплив екстракту (100 мг/кг та 1 г/кг) та настойки (1 та 5 мл/кг) надземної частини яглиці звичайної Aegopodium podagraria L. на фізичну працездатність, депресивність, когнітивні процеси у тестах плавання з навантаженням, екстраполяційного позбавлення і резерпінової депресії. Екстракт яглиці в дозі 100 мг/кг, але не 1 г/кг, достовірно підвищує час плавання з навантаженням (10% та 20%) у мишей-самців. У мишей-самок час плавання збільшується за використання навантаження 20% на тлі екстракту в обох дозах. Настойка яглиці не змінює результати даного тесту. Препарати яглиці неоднозначно впливають на результати тесту екстраполяційного позбавлення: екстракт та настойка не змінюють кількість мишей, що успішно виконують тест, однак достовірно знижується час позбавлення серед цих тварин (але не серед усієї групи) на тлі екстракту в дозах 100 мг/кг та 1 г/кг. Екстракт (100 мг/кг) достовірно знижує кількість щурів, які здійснюють пірнання, екстракт (1 г/кг) та настойка (1 мл/кг) збільшують час виконання даного тесту щурами. Екстракт та настойка не змінюють вираженість зниження температури тіла та блефароптозу, спричинених резерпіном у щурів. Отже, екстракт яглиці здатен підвищувати фізичну працездатність, чинити помірний позитивний вплив на когнітивні процеси в мишей (але не у щурів) без суттєвих змін депресивності. Настойка яглиці погіршує результати тесту екстраполяційного позбавлення в дозі 1 мл/кг, не змінює їх у дозі 5 мл/кг, не впливає на депресивність та фізичну працездатність в обох дозах.

ВЛИЯНИЕ ПРЕПАРАТОВ СНЫТИ ОБЫКНОВЕННОЙ *(AEGOPODIUM PODAGRARIA L.)* НА ФИЗИЧЕСКУЮ ВЫНОСЛИВОСТЬ, КОГНИТИВНЫЕ ФУНКЦИИ И ДЕПРЕССИВНОСТЬ ЖИВОТНЫХ

О.В.Товчига, С.Ю.Штрыголь

***Ключевые слова**: сныть обыкновенная (Aegopodium podagraria L.); экстракт; настойка; центральная нервная система; физическая работоспособность*

Исследовано влияние экстракта (100 мг/кг и 1 г/кг), а также настойки (1 и 5 мл/кг) надземной части сныти обыкновенной Aegopodium podagraria L. на физическую работоспособность, депрессивность, когнитивные процессы в тестах плавания с нагрузкой, экстраполяционного избавления и резерпиновой депрессии. Экстракт сныти в дозе 100 мг/кг, но не 1 г/кг, достоверно увеличивает длительность плавания с нагрузкой (10% и 20%) у мышей-самцов. У мышей-самок возрастание длительности плавания зарегистрировано при использовании нагрузки 20% на фоне экстракта в обеих дозах. Настойка сныти не изменяет результаты данного теста. Препараты сныти неоднозначно влияют на результаты теста экстраполяционного избавления: экстракт и настойка не влияют на количество мышей, которые успешно выполняют тест, но среди выполнивших тест животных время избавления достоверно снижается на фоне экстракта в дозах 100 мг/кг и 1 г/кг (без изменений показателя всей группы). Экстракт в дозе 100 мг/кг достоверно уменьшает количество крыс, которые осуществляют подныривание, экстракт (1 г/кг) и настойка (1 мл/кг) увеличивают время, необходимое для выполнения теста экстраполяционного избавления крысами. Экстракт и настойка сныти не изменяют выраженность понижения температуры тела и блефароптоза, вызванных резерпином у крыс. Таким образом, экстракт сныти способен повышать физическую работоспособность, оказывать умеренное позитивное влияние на когнитивные процессы у мышей (но не у крыс) без существенных изменений депрессивности. Настойка сныти ухудшает результаты теста экстраполяционного избавления в дозе 1 мл/кг, не изменяет их в дозе 5 мл/кг, не влияет на депрессивность и физическую работоспособность в обеих дозах.

АВТОРСЬКИЙ ПОКАЖЧИК СТАТЕЙ ЖУРНАЛУ “ВІСНИК ФАРМАЦІЇ” ЗА 2015 РІК

ДО 60-РІЧЧЯ ЗАВІДУВАЧА КАФЕДРИ ОРГАНІЧНОЇ ХІМІЇ НАЦІОНАЛЬНОГО ФАРМАЦЕВТИЧНОГО УНІВЕРСИТЕТУ, ДОКТОРА ХІМІЧНИХ НАУК, ПРОФЕСОРА ШЕМЧУКА ЛЕОНІДА АНТОНОВИЧА

15 лютого 2016 року виповнилось 60 років з дня народження завідувача кафедри органічної хімії Національного фармацевтичного університету, доктора хімічних наук, професора Шемчука Леоніда Антоновича.

Л.А.Шемчук народився у м. Чуднів Житомирської області. Після закінчення у 1973 р. середньої школи вступив до Харківського фармацевтичного інституту, який закінчив з відзнакою у 1978 р. Ще у студентські роки захопився органічним синтезом і пов'язав своє життя з наукою. Після служби в армії (1978-1980) повернувся до ХФІ на кафедру органічної хімії, де з 1 вересня 1980 року працював на посаді асистента і заочно навчався в аспірантурі (1980-1984), з 1986 до 2001 р. – доцент, з 2001 до 2015 р. – професор, з березня 2015 р. – завідувач кафедри органічної хімії.

У 1985 р. Л.А.Шемчук захистив дисертацію на здобуття наукового ступеня кандидата фармацевтичних наук на тему «Синтез, свойства и биологическая активность эфиров аренсульфонилоксаминовых кислот и продуктов их превращения» під керівництвом д-ра хім. наук, проф. П.О.Петюніна. У 1999 р. захистив докторську дисертацію «Синтез та хімічні перетворення похідних глутарамінових кислот і гетероциклічних сполук на їх основі», науковий консультант – академік НАН України, д-р фарм. наук, д-р хім. наук, проф. В.П.Черних. У березні 2004 р. Л.А.Шемчуку було присвоєне звання професора.

Професор Шемчук Л.А. – відомий вчений в області синтезу біологічно активних речовин, під керівництвом якого проводяться дослідження синтезу гетероциклічних сполук на основі нітрогеновмісних бінуклеофілів та дикарбонільних похідних, дослідження взаємодії функціоналізованих гетероциклічних систем з реактивом Гриньяра, вивчення їх хімічних властивостей, кінетичних та термодинамічних параметрів реакцій, встановлення впливу структурних особливостей та умов проведення реакції гетероциклізації на її напрямок, дослідження фармакологічної активності синтезованих сполук та створення на їх основі високоефективних і малотоксичних лікарських речовин. Він є автором понад 250 публікацій, з них понад 90 статей у наукових журналах, має 2 авторських свідоцтва СРСР, 5 патентів України. Підготував 6 кандидатів наук, був керівником 7 магістерських та 13 дипломних робіт, на сьогодні під його керівництвом виконується 4 кандидатські дисертації.

Як здобуток у навчально-методичній роботі – понад 30 видань. Він є співавтором навчальних посібників: «Руководство к лабораторным и семинарским занятиям по органической химии» для студентів фармацевтичних ВНЗ (факультетів) (1989 – рос., 1991 – укр. мовами), «Organic chemistry tests collection» (2008), «Organic chemistry. Short lecture course», «Органічна хімія. Тести з поясненнями», «Прикладна ІЧ-спектроскопія», «Applied infrared spectroscopy» (2014); «Органическая химия. Тесты с пояснениями», «Organic chemistry. Tests with explanations» (2015).

Професор Л.А.Шемчук є членом Вченої ради НФаУ, членом Спецради Д 64.605.01 з правом прийняття до розгляду та проведення захисту дисертацій на здобуття наукового ступеня доктора (кандидата) фармацевтичних наук за спеціальністю 15.00.02 «Фармацевтична хімія та фармакогнозія», куратором секції «Фармацевтична хімія» Проблемної комісії НФаУ, членом оргкомітетів Всеукраїнських та міжнародних конференцій, присвячених синтезу БАР.

За сумлінну працю, значний особистий внесок у підготовку спеціалістів фармації України та розвиток фармацевтичної і хімічної науки, професійну ерудицію, активну життєву позицію Л.А.Шемчук удостоєний звання «Заслужений викладач НФаУ», нагороджений Почесними грамотами МОЗ України, Українського хімічного товариства, Національного фармацевтичного університету, він є дипломантом обласного конкурсу «Вища школа Харківщини – кращі імена» (2001, 2002). У 2015 р. здобув Диплом стипендіата в галузі науки ім. М.О.Валяшка (з фармації).

Леонід Антонович – чуйна, інтелігентна, талановита, ерудована людина, якій притаманні найкращі людські риси – чуйність, скромність, доброта, постійне самовдосконалення знань і готовність поділитись ними з колегами і молоддю, він високопрофесіональний викладач органічної хімії і знаний науковець, який користується заслуженою повагою і авторитетом серед студентів і співробітників університету.

Адміністрація Національного фармацевтичного університету, колектив кафедри органічної хімії, студенти, аспіранти, колеги та друзі щиро вітають ювіляра, бажають йому міцного здоров'я, великого людського щастя, сімейного добробуту. Нехай любов до життя, справжній талант та безмежна ерудиція і надалі ведуть його до успіхів, досягнення задуманого та нових творчих злетів у науково-педагогічній діяльності.

ЗМІСТ / CONTENTS / СОДЕРЖАНИЕ

СИНТЕЗ ТА АНАЛІЗ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН

ТЕХНОЛОГІЯ ЛІКАРСЬКИХ ПРЕПАРАТІВ

ОРГАНІЗАЦІЯ ТА ЕКОНОМІКА ФАРМАЦІЇ

ЕКСПЕРИМЕНТАЛЬНА ТА КЛІНІЧНА ФАРМАКОЛОГІЯ

Адреса для листування: 61002, м. Харків, вул. Пушкінська, 53, Національний фармацевтичний університет, редакція журналу "Вісник фармації", тел./факс (057) 706-30-63. E-mail: newspharm-journal@nuph.edu.ua, press@nuph.edu.ua. Сайт журналу: http://nphj.nuph.edu.ua
Передплатні індекси: для індивідуальних передплатників — 74102; для підприємств — 74103.

Свідоцтво про державну реєстрацію серія КВ №14938-3910ПР від 04.02.2009 р.

Підписано до друку 11.03.2016 р. Формат 60x84 1/8. Папір офсетний. Друк ризографія.
Умовн. друк. арк. 10,23. Обліков.-вид. арк. 11,87. Тираж 100 прим.

Редактори О.Ю.Гурко, А.Л.Краснікова; комп'ютерна верстка О.О.Воробйов.