19. Matematychni metody i modeli rynkovoi ekonomiky: textbook / Klebanova T. S., Kyzym M. O., Cherniak O. I. et. al. Kharkiv: VD "INZhEK", 2009. 456 p.
20. Prokopenko I. F., Hanin V. I., Petriaieva Z. F. Kurs ekonomichnoho analizu: textbook / ed. by Prokopenko I. F. Kharkiv: Lehas, 2004. 384 p.

Дата надходження рукопису 19.03.2019

Karina Golubtsova, Department Processes and Apparatuses of Chemical and Pharmaceutical Industries, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002
E-mail: paft@nuph.edu.ua

Rita Sahaidak-Nikitiuk, Doctor of Pharmaceutical Sciences, Head of Department, Department of Processes and Apparatuses of Chemical and Pharmaceutical Industries, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002
E-mail: sagaidak_rita@ukr.net

Natalia Demchenko, PhD, Associate Professor, Department of Management and Administration, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002
E-mail: demchenata@ukr.net

UDC 547.789.06:543.544.5.068.7
DOI: 10.15587/2519-4852.2019.165763

DEVELOPMENT OF METHOD OF QUANTITATIVE DETERMINATION OF CARDIAZOL SUBSTANCE WITH USING HIGHLY EFFICIENT LIQUID CHROMATOGRAPHY

© I. Drapak, B. Zimenkovsky. I. Bezruk, L. Ivanauskas, L. Perekoda, S. Harna

Мета. *Розробка методики кількісного визначення субстанції Кардіазол з використанням високоефективної рідинної хроматографії.*
Матеріали і методи*. Методом високоефективної рідинної хроматографії (ВЕРХ) проводили кількісне визначення субстанції Кардіазол ([3-алліл-4-(4^1-метоксифеніл)-3Н-тіазол-2-іліден]-(3^2-трифлуорометилфеніл)аміну гідробромід), використовуючи систему ShimadzuNexeraX2 LC-30AD (Shimadzu, Японія). В роботі використовувалися ацетонітрил класу HPLC (Sigma-AldrichGmbH, Швейцарія), інші хімічні речовини та розчинники були аналітичного сорту. Досліджувану субстанцію Кардіазол розчиняли в ацетонітрилі з кінцевою концентрацією 400 мкг/мл.*
Результати і обговорення. *Виявлені наступні оптимальні умови хроматографічного розподілу: колонка С8 (250*4,6 мм; швидкість рухомої фази 1 мл /хв; температура термостату колонки 35 ºС; довжина хвилі детектування 300 нм, час утримування досліджуваної сполуки становить 13,9 хв. Продуктивність колонки була визначена для її основних показників, таких як кількість теоретичних тарілок(більше 65000) і коефіцієнт симетрії (близько 1,00). Методика була валідована згідно з рекомендаціями ДФУ. Методику було апробовано на вплив різних факторів, таких як, швидкість потоку, склад рухомої фази та температура термостату колонки. Встановлено, що вплив цих факторів є незначущим та не впливає на результати, отримані за цією методикою.*
Висновки. *На основі методу високоефективної рідинної хроматографії розроблено аналітичну методику кількісного визначення субстанції кардіопротекторної дії Кардіазолу. Стандартизовано умови проведення хроматографічного аналізу (ВЕРХ). Встановлено вимоги до тесту «Перевірка придатності хроматографічної системи». Статистична обробка результатів експерименту свідчить, що відносна невизначеність середнього результату знаходиться у допустимих межах. Розроблена методика буде використана для подальшого дослідження речовини як компонента різних лікарських форм*
Ключові слова: *Кардіазол, кардіопротекторна активність, аналіз, методика кількісного визначення, високоефективна рідинна хроматографія*

1. Introduction

In the medicine and pharmacy industry, numerous developments are being carried out on original multidirectional drugs, among which special attention is drawn to drugs that can be used to treat cardiovascular diseases. The urgency of the search for biologically active compounds for the treatment of cardiovascular diseases is due to the fact that these diseases are the main cause of

mortality, disability, disability of the population all over the world. According to the WHO, Ukraine is the number 1 in Europe and number 2 in the world in the ranking of cardiovascular mortality vascular diseases. Cardiovascular diseases account for 47 % of all deaths in European countries [1, 2]. Previous studies have proven the prospect of finding potential biologically active substances in a number of derivatives of 1,3-thiazole [3, 4]. Based on previous studies using modern methods of computer chemistry purposefully designed and synthesized a novel substance [3-Allil-4-(4^1-methoxyphenyl)-3H-thiazole-2-ylidene]-(3^2-trifluoro-methylphenyl)amine hydrobromide (Cardiazole) exhibiting cardioprotective, anti-inflammatory, analgesic, hypolipidemic and antioxidant action (Fig. 1) [5, 6].

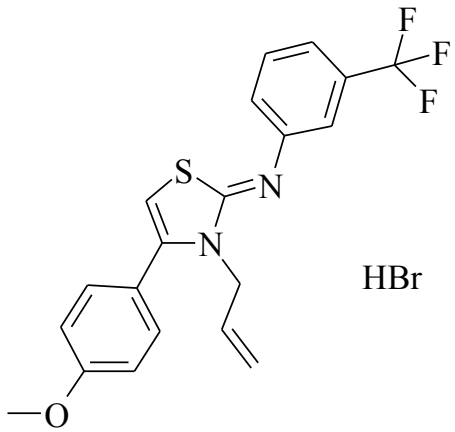

Fig. 1. Crystalline [3-Allil-4-(4^1-methoxyphenyl)-3H-thiazole-2-ylidene]-(3^2-trifluoro-methylphenyl)amine hydrobromide

The synthesized compound in the experimental in vitro experiment on isolated rings of the aorta of the laboratory rats was more effective in reducing the contractile response of the smooth muscles to hypoxia compared with L-carnitine and meldonium. The use of a compound in white rats on the model of adrenal infarction has a pronounced and higher cardioprotective effect than the meldonium. The strain is patented, proposed for further preclinical studies under the conditional name Cardiazole [7]. Therefore, the development of an optimal, high-precision, reproducible method of quantitative determination of Cardiazole is very relevant and necessary.

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues

The introduction of a new medicinal product or substance into medical practice is impossible without the development of methods for its analysis [8, 9]. The development of modern, unified, efficient and simple methods for the analysis of substances is one of the main tasks of pharmaceutical science.

Modern understanding of quality assurance approaches is based on the concept of quality assurance of medicines from the pharmaceutical development and research stage through proper manufacturing, quality control, storage, sales and provision of information to doctors and patients [10, 11].

3. Analysis of recent studies and publications in which a solution of the problem are described and to which the author refers

One of the most important stages of the introduction of a new medicinal product or substance into medical practice is the development of quality control methods, in particular, the methods of quantitative determination. Such studies are needed to standardize the substance, to study pharmacokinetics and pharmacodynamics, bioequivalence, pharmaceutical development of dosage forms on its basis.

In previous studies of scientists for quantitative determination of 1,3-thiazole derivatives a method of potentiometric titration in a non-aqueous solvent medium [12], fluorescence spectroscopy [13] was used. The researchers also developed a simple and sensitive spectrophotometric method for the determination of 1,3-thiazole cephalosporins [14]. But the reference in the literature for the use for quantitative determination of 1,3-thiazole derivatives of the method of high-performance liquid chromatography is not numerous, therefore, we consider them to be relevant.

4. The field of research considering the general problem, which is described in the article

According to the recommendations of the SPHU, the quantitative determination of a substance containing atoms of Nitrogen may be carried out by the method of determination of nitrogen after mineralization using sulfuric acid, which is described in the general monograph of SPHU 2.5.9. But the above method is inappropriate for Sardiazole dosage forms, as auxiliary substances may interfere with the determination of the substance. To solve this problem, it is advisable to apply new, more modern methods of analysis; including high-performance liquid chromatography (HPLC), which is one of the most modern high-sensitivity, universal, automated methods of analysis. HPLC can be applied both for identification and for the quantitative determination of drugs, allows results to be obtained quickly, with high accuracy and reliability [15, 16]. Thus, the development of a method for quantifying the substance of Cardiazole using HPLC for further pharmaceutical development is completely necessary and appropriate.

5. Formulating the goals (tasks) of the article

In order to prepare the substance for introduction, we have developed a method for quantitatively determining the substance of Cardiazole using HPLC.

6. Presentation of the main research material (methods and objects) with the justification of the results

Liquid chromatography separation was performed using a Shimadzu Nexera X2 LC-30AD HPLC system (Shimadzu, Japan) composed of a quaternary pump, an on-line degasser, a column temperature controller, the SIL-30AC autosampler (Shimadzu, Japan); the CTO-20AC thermostat (Shimadzu, Japan) as well as the SPD-M20A diode array detector (DAD). Another instruments such as Ultrasonic Cleaner Set for ultra-sonication using (Wise Clean WUC-A06H, Witeg Labortechnik GmbH, Germany), Libra UniBloc AUW120D (Shimadzu Ana-

lytical Scale, Japan); class A analytical vassals that meets requirements of the SPhU (SPhU, 2015) were used in the investigation. HPLC grade acetonitrile (Sigma-Aldrich GmbH, Switzerland), were used in the analysis work. HPLC grade water was obtained from a water purifying system (Millipore, Bedford, MA, USA). Other chemicals and solvents were of analytical grade.

The method of quantitative determination of Cardiazole using HPLC was performed on the column ACE 5 C8 (250 * 4.6 mm, particle size µm). A binary system of mobile phase solvents was used: solvent A (water) and solvent B (acetonitrile). The following profile of the linear gradient elution was used: 55 % A / 45 % B - 0 min, 55 % A / 45 % B - 3 min, 20 % A / 80 % B - 7 min, 20 % A / 80 % B-14 min, 55 % A / 45 % B - 16 minutes and 55 % A / 45 % B - 20 minutes. The flow rate was 1 ml / min, the volume of the injection was 10 µL. The column temperature was constant at 35 °C. Chromatograms were recorded at 300 nm. Under the proposed conditions, the retention time of the investigated component is 13.9 min.

Method

Test solution. 40 mg (precisely weighed amount) of the substance Cardiazole ([3-Allil-4- (41-methoxyphenyl) -3H-thiazole-2-ylidene] - (32-trifluoromethylphenyl) amine hydrobromide) is placed in a 100 ml volumetric flask, dissolved in 30 ml of acetonitrile P and bring the volume of the solution to the mark with the same solvent, mix thoroughly. Filter through a membrane filter with a pore diameter of no more than 0.45 µm. The solution is freshly prepared.

Comparison solution. 40 mg (precisely weighed amount) of Cardiazole substance are placed in a 100 ml volumetric flask, dissolved in 30 ml of acetonitrile P, and the volume of the solution is adjusted to the mark with the same solvent, carefully mixed. Filter through a membrane filter with a pore diameter of no more than 0.45 µm. The solution is freshly prepared.

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

– a column measuring 250×4.6 mm, filled with octylsilyl silica gel for chromatography P (for example, ACE C8, 150×4.6 mm, YMC company) with a pre-column having a particle size of 5 µm for which the chromatographic system's suitability conditions are fulfilled;

– speed of the mobile phase: 1.0 ml/min;
– column temperature: 35 °C;
– Detection at wavelength: 300 nm;
– volume of injection: 10 µl;
– mobile phase A: water for chromatography P;
– mobile phase B: acetonitrile P;
– gradient program (shown in Table 1).

Table 1

The gradient chromatographic program

Time, min	Mobile phase A, %	Mobile phase B, %
0:00	55	45
03:00	55	45
07:00	20	80
14:00	20	80
16:00	55	45
20:00	55	80

Exit time of the main peak is 13.89 min. Fig. 2 shows the chromatogram of the test solution Cardiazole.

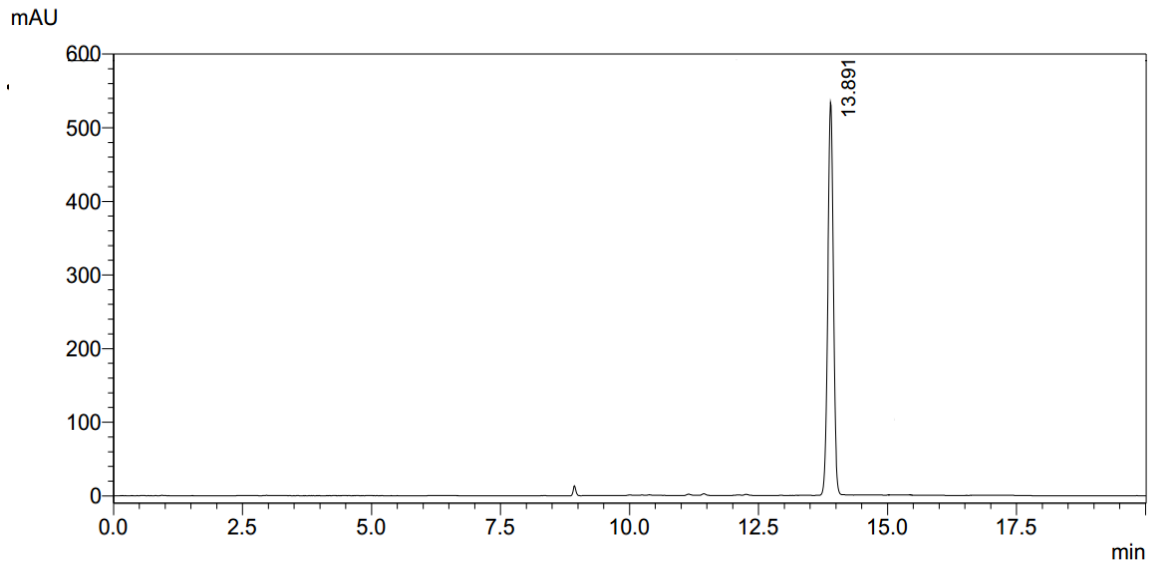

Fig. 2. The chromatogram of the test solution Cardiazole

The method was validated in accordance with the recommendations of the SPHU [17, 18]. The chromatographic system is considered to be suitable if the following conditions are met for the comparison solution:

– the efficiency of the chromatographic column, calculated on the peak of the main substance, must be at least 3000 theoretical plates;

– the coefficient of peak symmetry should be in the range from 0.8 to 1.5;

– the relative standard deviation for peak areas, should not exceed 1.0 %, calculated on the results of 5 injections.

Characteristics of the chromatographic system for the comparison solution of Cardiazole are given in Table 2

Table 2

Characteristics of the chromatographic system for the comparison solution

Substance	Efficiency (number of theoretical plates)	Symmetry factor	Relative standard deviation, %
Cardiazole	67504	0.99	0.02

Forecast of uncertainty of analysis results

The maximum permissible absolute relative uncertainty of the method of analysis of the substance ΔAS % is related to the symmetric tolerances of the content of the analyzed substance in accordance with the specification (B). That is:

$$\Delta AS \le \frac{B_н - B_L}{2} \times 0,32$$

In the substance of Cardiazole, the quantitative content of its components should be within ±5 %, therefore:

$$\Delta AS \le \frac{95-105}{2} \times 0,32 = 1,6\ \%$$

Calculation of uncertainty of sample preparation is given in Table 3.

Table 3

Calculation of sample preparation uncertainty (Δ_{sr})

Operation of sample preparation	Value	Uncertainty, (Δ), %
Test solution		
Weigh (m)	40 mg	0.5
Uncertainty of weighing	0,2 mg	
Bringing to volume	100 ml	0.12
Comparison solution		
Weigh (m)	40 mg	0.25
Uncertainty of weighing	0,2 mg	
Bringing to volume	100 ml	0.12
Complete uncertainty of sample preparation Δsp %		**0.73**
*Uncertainty of the final analytical operation Δ_{FAO} (liquid chromatography)**		**1.35**
Complete uncertainty of the method of analysis Δ_{AS} % $\Delta_{AS}\% = \sqrt{(\Delta sp\ \%)^2 + (\Delta_{FAO}\%)^2}$		**1.53**

The determination of the uncertainty of the final analytical operation Δ_{FAO} is carried out for the test solution and the solution of the comparison. When calculating confidence intervals, one-sided Student's coefficient is used for the probability of 95 % and the corresponding number of degrees of freedom. Trust intervals for the solution for comparison and the test solution are calculated for an average of 5 results (the maximum number of measurements according to drug MQC).

$$\Delta_{FAO}^{cm} = \frac{1}{\sqrt{5}} \times t(95\ \%,\ n-1) \times RSD$$

$$\Delta_{FAO}^{smp} = \frac{1}{\sqrt{5}} \times t(95\ \%,\ n-1) \times RSD$$

According to the requirements of the applicability of the chromatographic system of the determination method, the relative standard deviation of the five parallel determinations must be no more than 1.0 %. When n = 5, t (95 %, n-1) = 2.1318:

$$\Delta_{FAO}^{cm} = \frac{1}{\sqrt{5}} \times 2.1318 \times 1.0\ \% = 0.9534$$

$$\Delta_{FAO}^{smp} = \frac{1}{\sqrt{5}} \times 2.1318 \times 1.0\ \% = 0,9534$$

Total uncertainty of the final analytical operation:

$$\Delta_{FAO} = \sqrt{(\Delta_{FAO}^{smp})^2 + (\Delta_{FAO}^{smp})^2} = 1.35$$

Complete uncertainty of the method of analysis *Δ_{AS} %:*

$$\Delta_{AS} = \sqrt{(\Delta_{sp})^2 + (\Delta_{sp})^2} = 1.50\ \%$$

$$\Delta_{AS} = \sqrt{(\Delta_{sp})^2 + (\Delta_{sp})^2} = 1.49\ \%$$

Thus, the complete uncertainty of the analysis method Δ_{AS} % is calculated to be less than max Δ_{AS}

(1.53 % <max Δ_{AS}=1.6 %;), which meets the requirements for this parameter.

Consequently, the uncertainty of sample preparation and analysis as a whole should provide sufficient accuracy of measurement.

Thus, the developed method of quantitative determination of Cardiazole meets the requirements of "Suitability of the chromatographic system" according to the parameters: the efficiency of the chromatographic column, the coefficient of separation of peaks on the chromatogram, the rate of asymmetry of the peak. The proposed method allows reliable identification of Cardiazole and quantify it.

7. Conclusions from the conducted research and prospects for further development of this field

1. Based on the method of high-performance liquid chromatography, an analytical method for the quantitative determination of Cardiazole has been developed.

2. The conditions for chromatographic analysis (HPLC) are standardized. Requirements for the test "Checking the suitability of the chromatographic system" are established. The statistical processing of the results of the experiment shows that the relative uncertainty of the average result is within the permissible limits.

3. The developed method for quantitative determination of Cardiazole will be used for further study of matter as a component of various dosage forms.

References

1. Comparative characteristics of the cardiovascular disease prevention in Ukraine and Europe according to EUROASPIRE IV data: a hospital line / Kovalenko V. M., Dolzhenko M. M., Nesukai Ye. H., Diachenko Ya. S. // Arterial'naya gipertenziya. 2016. Issue 1. P. 41–80.

2. The top 10 causes of death // WHO. URL: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death

3. Synthesis of 2-(4-aryl(adamantyl)-2-phenyliminothiazol-3-yl)-ethanol derivatives and prediction of their biological activity / Yeromina H. O., Drapak I. V., Perekhoda L. O., Yaremenko V. D., Demchenko A. M. // Der Pharma Chemica. 2016. Vol. 8, Issue 3. P. 64–70.

4. The antioxidant properties of 1-[2-(R-phenylimino)-4-methyl-3-(3-[morpholine-4yl]propyl)-2,3-dihydro-1,3-thiazol-5-yl]ethane-1-one derivatives under conditions of artificial oxidative stress in vitro / Perekhoda L., Yeromina H., Drapak I., Kobzar N., Smolskiy O., Demchenko N. // Saudi Journal of Medical and Pharmaceutical Sciences. 2017. Vol. 3, Issue 1. P. 55–59.

5. The use of the docking studies with the purpose of searching potential cardioprotectors / Drapak I., Perekhoda L., Vynogradova O., Protopopov M., Suleiman M., Sych I., Kobzar N., Kiz O. // Pharmacia. 2018. Issue 2. P. 40–46.

6. Synthesis and in silico research of derivatives of 3-allyl-4-(R-phenyl)-N-(R1-phenyl)thiazole-2-imine / Perekhoda L. O., Drapak I. V., Suleiman M. M., Sych I. A., Yaremenko V. D. // Der Pharma Chemica. 2017. Vol. 9, Issue 13. P. 95–98.

7. Hidrobromid [3-alil-4-(41-metoksyfenil)-3N-tiazol-2-iliden]-(32-tryfluorometylfenil)aminu, shcho maie kardioprotektorni vlastyvosti: Pat. No. 122197 UA / Herashchenko I. V., Drapak I. V., Demchenko A. M., Perekhoda L. O., Niektiehaiev I. O. No. u201707267; declareted: 10.07.2017; published: 26.12.2017, Bul. No. 24.

8. Overview of the European Medicines Agency's Development of Product-Specific Bioequivalence Guidelines / O' Sullivan J., Blake K., Berntgen M., Salmonson T., Welink J. // Clinical Pharmacology & Therapeutics. 2018. Vol. 104, Issue 3. P. 539–545. doi: https://doi.org/10.1002/cpt.957

9. Non-clinical studies in the process of new drug development – Part II: Good laboratory practice, metabolism, pharmacokinetics, safety and dose translation to clinical studies / Andrade E. L., Bento A. F., Cavalli J., Oliveira S. K., Schwanke R. C., Siqueira J. M. et. al. // Brazilian Journal of Medical and Biological Research. 2016. Vol. 49, Issue 12. doi: https://doi.org/10.1590/1414-431x20165646

10. Quality in the pharmaceutical industry – A literature review / Haleem R. M., Salem M. Y., Fatahallah F. A., Abdelfattah L. E. // Saudi Pharmaceutical Journal. 2015. Vol. 23, Issue 5. P. 463–469. doi: https://doi.org/10.1016/j.jsps.2013.11.004

11. Baumann M., Baxendale I. R. The synthesis of active pharmaceutical ingredients (APIs) using continuous flow chemistry // Beilstein Journal of Organic Chemistry. 2015. Vol. 11. P. 1194–1219. doi: https://doi.org/10.3762/bjoc.11.134

13. Rozrobka metodyky kilkisnoho vyznachennia substantsiyi hidrobromidu (3-etyl-4-fenil-3N-tiazol-2-iliden)- [4-(6,7,8,9-tetrahidro-5N-[1,2,4]tryazolo[4,3-a]azepin-3-yil)fenil]aminu / Yeromina H. O., Yeromina Z. H., Sych I. A., Perekhoda L. O. // Upravlinnia yakistiu v farmatsiyi: mat. XI nauk.-prakt. konf. Kharkiv, 2017. P. 64.

14. Shim J.-S. K., Tolan J. W., Fink D. W. Fluorometric Determination of Thiazole-Containing Compounds // Journal of Pharmaceutical Sciences. 1980. Vol. 69, Issue 3. P. 275–279. doi: https://doi.org/10.1002/jps.2600690308

15. Quantitative determination of some thiazole cephalosporins through complexation with palladium (II) chloride / El-Walily A. F. M., Gazy A. A., Belal S. F., Khamis E. F. // Journal of Pharmaceutical and Biomedical Analysis. 2000. Vol. 22, Issue 2. P. 385–392. doi: https://doi.org/10.1016/s0731-7085(99)00281-2

16. High performance liquid chromatography in pharmaceutical analyses / Nikolin B., Imamović B., Medanhodžić-Vuk S., Sober M. // Bosnian Journal of Basic Medical Sciences. 2004. Vol. 4, Issue 2. P. 5–9. doi: https://doi.org/10.17305/bjbms.2004.3405

17. Pakhomov V. P. Khromatohrafiya u khimiko-farmatsevtychnykh doslidzhenniakh // Khymyko-farmatsevtycheskyi zhurnal. 2003. Vol. 37, Issue 8. P. 55–56.

18. Derzhavna Farmakopeia Ukrainy. Vol. 1. 2-e vyd. Kharkiv: DP «Ukrainskyi naukovyi farmakopeinyi tsentr yakosti likarskykh zasobiv», 2015. 1128 p.

19. European Pharmacopoeia. European Directorate for the Quality of Medicines & Health Care. 9-th ed. Strasbourg, 2017.

Дата надходження рукопису 05.03.2019

Drapak Iryna, PhD, Associate Professor. Department of General, Bioinorganic, Physical and Colloidal Chemistry, Danylo Halytsky Lviv National Medical University, Pekarska str., 69, Lviv, Ukraine, 79010
E-mail: iradrapak@ukr.net

Zimenkovsky Borys, Doctor of Pharmaceutical Sciences, Professor, Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, Pekarska str., 69, Lviv, Ukraine, 79010
E-mail: d35.600.02@meduniv.lviv.ua

Bezruk Ivan, Postgtaduate student, Department of Pharmaceutical Chemistry, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraina, 61002
E-mail: vania.bezruk@gmail.com

Liudas Ivanauskas, PhD, Professor, Department of Analytical and Toxicological Chemistry, Lithuanian University of Health Sciences, A. Mickevičiaus g. 9, Kaunas, Lithuania, LT 44307
E-mail: liudas.ivanauskas@lsmuni.lt

Perekhoda Lina, Doctor of Pharmaceutical Sciences, Professor, Department of Medicinal Chemistry, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002
E-mail: linaperekhoda@ukr.net

Harna Svitlana, Doctor of Pharmaceutical Sciences, Professor, Department of Quality, Standartization and Sertification of Drugs, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002
E-mail: garnaya57@gmail.com