UDC 543.51:547.792'292-38 DOI: 10.14739/2310-1210.2015.5.53774

V. A. Salionov, B. A. Varynskyi, V. V. Parchenko

Mass-spectrometric fragmentation of sodium 2-(4-methyl-5-(thiophene-2-yl)-4*H*-1,2,4-triazole-3-ylthio)acetate

Zaporizhzhia State Medical University

Key words: *1,2,4-triazoles, Mass Spectrometry, High Performance Liquid Chromatography, Ionization In Electrospray.*

The study of physical and chemical characteristics and establishing patterns of mass spectrometric fragmentation are the actual tasks of modern pharmaceutical science, have both scientific interest and practical importance.

Aim. The purpose of our experiment was to confirm the identity and study patterns of mass spectrometric decomposition for sodium 2-(4-methyl-5-(thiophene-2-yl) -4*H*-1,2,4-triazoles-3-ilthio) acetate.

Methods and results. The study was carried out on the apparatus LC MS / MS: LTQ Orbitrap. After completing MS scan, it has been established that the maximum peak in the chromatogram corresponds protonated ion 2-(4-methyl-5-(thiophene-2-yl)-4*H*-1,2,4-triazoles-3-ylthio) acetic acid m/z 256.0208, and empirical formula test compound – $C_9H_9N_3O_2S_2$. MS/MS MH^+ analysis showed that protonated molecule of the 2-(4-methyl-5-(thiophene-2-yl)-4*H*-1,2,4-3-triazole-3-ylthio)acetic acid under voltage in contact with molecules of helium in the collision cell was disintegrated from m/z 256.0208 into some fragments. The first time the mass spectrometric investigation sodium 2-(4-methyl-5-(thiophene-2-yl)-4*H*-1,2,4-triazoles-3-ylthio) acetate has been undertaken.

Conclusions. It has been established that the fragmentation of the molecule initially occurs with the dissociation of water molecule from carboxyl group. Under the influence of gas the next to come off may be a carboxyl group. In course of further dissociation the bond between the sulfur atom and acetic residue is destroyed or disruption of a covalent bond between sulfur and carbon of 1,2,4-triazole occurs.

Мас-спектрометричний розпад молекули натрій 2-(4-метил-5-(тіофен-2-іл)-4Н-1,2,4-тріазол-3-ілтіо)ацетату

В. О. Саліонов, Б. О. Варинський, В. В. Парченко

Вивчення фізико-хімічних характеристик, а також встановлення закономірностей мас-спектрометричного розпаду є актуальними завданнями сучасної фармацевтичної науки, мають як науковий інтерес, так і практичну значущість. Мета експерименту – підтвердження індивідуальності та дослідження мас-спектрометричного розпаду для молекули натрій 2-(4-метил-5-(тіофен-2-іл)-4Н-1,2,4-тріазол-3-ілтіо)ацетату. Дослідження виконали на приладі *LC MS/MS*: LTQ Orbitrap. Після виконання МС сканування встановили, що максимальний пік на хроматограмі відповідає іону з m/z 256,0208, а емпірична формула досліджуваної сполуки – $C_9H_9N_3O_2S_2$. MS/MS аналіз 2-(4-метил-5-(тіофен-2-іл)-4Н-1,2,4-тріазол-3-ілтіо)ацетатної кислоти (MH^+ з m/z 256,0208) показав, що під дією напруги при зіткненні з молекулами гелію в комірці зіткнень відбувається розпад молекули на деякі фрагменти. Встановили, що руйнування молекули спочатку здійснюється з відривом молекули води від карбоксильної групи. Під впливом зіткнення з газом наступною може відриватися карбоксильна група. При наступному розпаді руйнується зв'язок між атомом сульфуру та оцтовим залишком або ж відбувається розрив ковалентного зв'язку між сульфуром і вуглецем 1,2,4-тріазолу.

Ключові слова: *1,2,4-тріазол, мас-спектрометрія, високоефективна рідинна хроматографія, іонізація в електроспреї.*

Запорізький медичний журнал. – 2015. – №5 (92). – С. 93–96

Масс-спектрометрический распад молекулы натрий 2-(4-метил-5-(тиофен-2-ил)-4Н-1,2,4-триазол-3-илтио)ацетата

В. А. Салионов, Б. А. Варинский, В. В Парченко

Изучение физико-химических характеристик, а также установление закономерностей масс-спектрометрического распада являются актуальными задачами современной фармацевтической науки, имеют как научный интерес, так и практическую значимость. Целью нашего эксперимента было подтверждение индивидуальности и исследование масс-спектрометрического распада для молекулы натрий 2-(4-метил-5-(тиофен-2-ил)-4Н-1,2,4-триазол-3-илтио)ацетата. Исследование проведено на приборе *LC MS/MS*: LTQ Orbitrap. После выполнения МС сканирования установлено, что максимальный пик на хроматограмме соответствует иону с m/z 256,0208, а эмпирическая формула исследуемого соединения – $C_9H_9N_3O_2S_2$. MS/MS анализ 2-(4-метил-5-(тиофен-2-ил)-4Н-1,2,4-триазол-3-илтио) ацетатной кислоты (MH^+ с m/z 256,0208) показал, что под действием напряжения при столкновении с молекулами гелия в ячейке столкновений проходит распад молекулы на некоторые фрагменты. Установлено, что разрушение молекулы сначала проходит с отрывом молекулы воды от карбоксильной группы. Под влиянием столкновения с газом следующей может отрываться карбоксильная группа. При дальнейшем распаде разрушается связь между атомом серы и уксусным остатком или же происходит разрыв ковалентной связи между серой и углеродом 1,2,4-триазола.

Ключевые слова*: 1,2,4-триазол, масс-спектрометрия, высокоэффективная жидкостная хроматография, ионизация в электроспрее.*

Запорожский медицинский журнал. – 2015. – №5 (92). – С. 93–96

During several recent decades there was found a big amount of information considering the synthesis, physical-chemical and biological features of the 1,2,4-triazole derivatives. The authors of the scientific works pay a particular attention to salts of the 1,2,4-triazole-3-thio acetic acids. The reason is that the compositions of this class are mainly water-soluble, and the pharmacological function is displayed by both anion and cation. Thus, domestic preparations which were applied into the pharmacological («Thiox») [7] and veterinary («Trifuzol», «Avestim») [5,9] practices are salts of the 4-R-

5-R_1-1,2,4-triazole-3-thioacetic acid due to their constitution.

It is necessary to mention that the correctness of the established constitution of the biologically active substance, parameters of the clarity, possibility of quantitative and qualitative measurement give premises to the further implementation of this substance into the medical or veterinary practice.

In this way, the examination of physical-chemical characteristics and the establishment of regularities of the mass-spectrometric disintegration are urgent tasks for a present-day pharmacological science and they represent both scientific and practical importance [2,3,8,11].

The aim of our experiment was to confirm the individuality and investigation of the mass-spectrometric fragmentation for the sodium 2-(4-methyl-5-(thiophene-2-yl)-4*H*-1,2,4-triazole-3-ylthio)acetate, which has a high index of adaptogenic activity [1] and represents a great interest for the further preclinical trial.

Data and methods

The sodium 2-(4-methyl-5-(thiophene-2-yl)-4*H*-1,2,4-triazole-3-ylthio)acetate substance for the experiment was taken with the usage of the methodology [10] which composition was established with the help of chromatography-mass spectrometric analyses, proton nuclear magnetic resonance and infrared spectroscopy.

The research was made on the *LC MS/MS*: LTQ Orbitrap device.

The scanning during the main ion's SCAN1 mass identification - m/z 100.0000-500.0000, the scanning after the SCAN2 fragmentation - m/z 50.0000-260.0000.

The sample for the experiment was received by dilution of the sodium 2-(4-methyl-5-(thiophene-2-yl)-4*H*-1,2,4-triazole-3-ylthio)acetate in the water: methanol (1:1) with the solution concentration 100 ng/ml. Elution time – 6 min.

Software. Software package Xcalibur™ Software. Software package ChemBioOffice 2012.

Results and their discussion

On the first stage of the experiment our aim was to prove the composition of the examined substance. After the MC scanning (SCAN1), it was found that the maximal peak on the chromatogram corresponds to ion with m/z 256.0208, and empirical formula of the examined substance – $C_9H_9N_3O_2S_2$.

This phenomenon may be explained by the fact that in conditions of elution in acid environment as a result of the reaction with the acid sodium 2-(4-methyl-5-(thiophene-2-yl)-4*H*-1,2,4-triazole-3-ylthio)acetate with the molecule mass 277.30 g/mol transforms into the 2-(4-methyl-5-(thiophene-2-yl)-4*H*-1,2,4-triazole-3-ylthio)acetic acid, which in the protonated form has mono isotopic weight 256.0208 (*fig. 1*) [4,6]. The presence of sulfur was proved by the content of the big amount of its stable isotope ^{34}S (A+2), which is 9.86% from the ion's mono isotopic weight 256.0208, with the mass defect -0.0042, which is typical for this chemical element.

The next step of our research was the conduction of the fragmentation of the protonated 2-(4-methyl-5-(thiophene-2-yl)-4*H*-1,2,4-triazole-3-ylthio)acetate acid molecule (MH^+) with the further MS/MS analyses of the received practical values and theoretical calculations.

MS/MS analyses MH^+ (SCAN2) showed that under the voltage of collision with helium molecules in the cell of collision the molecule disintegrate into several fragments (*fig. 2*). The

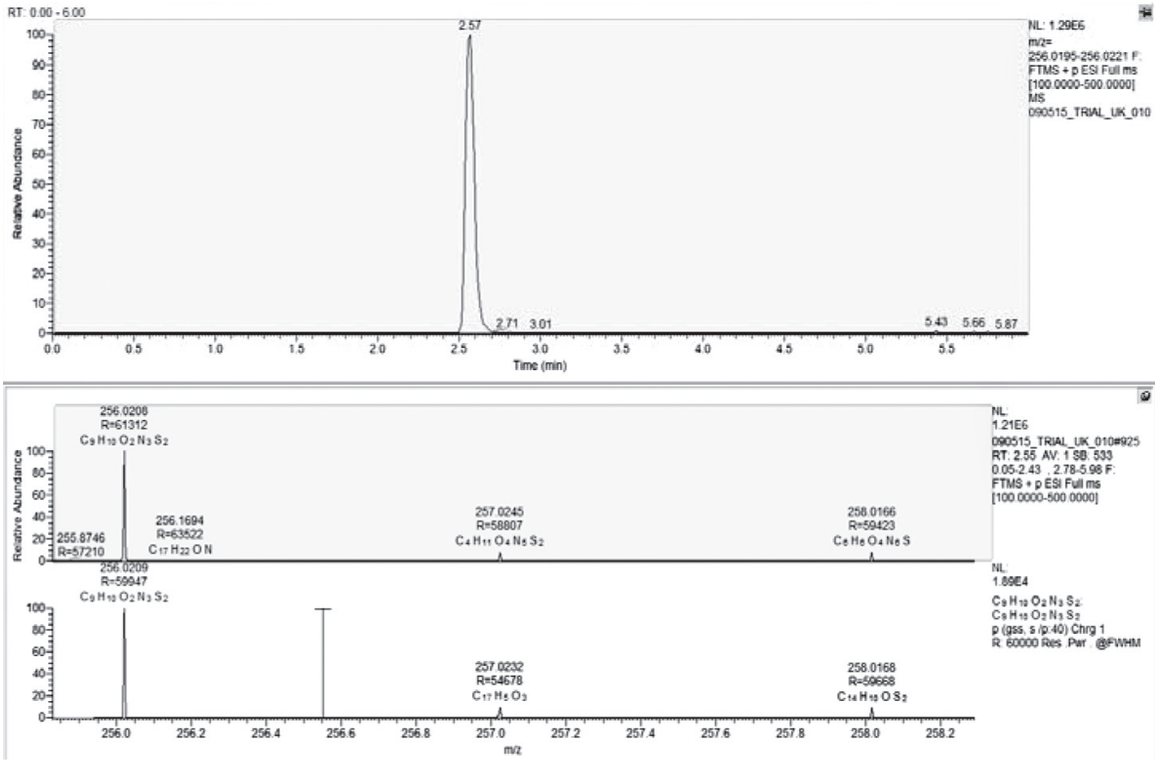

Fig.1. MS scanning of the sodium 2-(4-methyl-5-(thiophene-2-yl)-4*H*-1,2,4-triazole-3-ylthio)acetate.

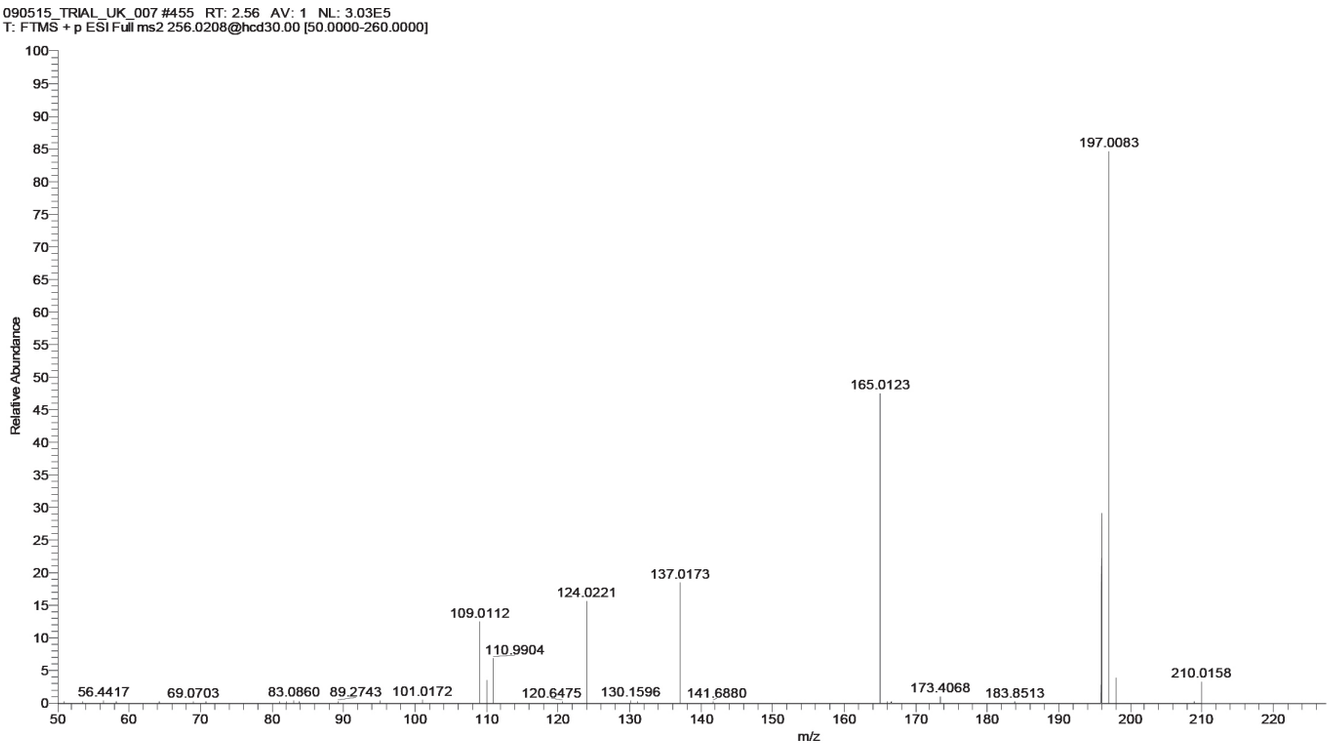

Fig. 2. MS/MS spectrum of the 2-(4-methyl-5-(thiophene-2-yl)-4*H*-1,2,4-triazole-3-ylthio) acetate.

formation of the molecule's fragment with m/z 238.0111 and molecule formula $C_9H_8N_3OS_2^+$ can affirm the initial molecule's loss of water (*fig. 3*). The next fragment with m/z 210,0154 ($C_8H_8N_3S_2^+$) may be created when the initial molecule will lose carboxyl group. Fragmental ion with m/z 197.0083 ($C_7H_7N_3S_2^+$) affirms that there is a destruction of the bond between the sulfur atom and the methyl atom of carbon, in other words proceeds the split of the lateral chain -CH_2COOH, whereas the formation of the ion with m/z 195.9997 ($C_7H_6N_3S_2^+$) affirms the loss of the one atom of the hydrogen in the ion with m/z 197.0083 and regrouping it in the molecule.

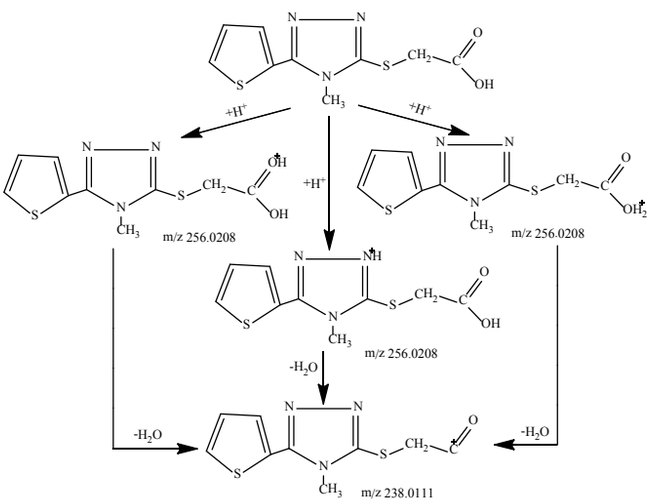

Fig. 3. Mechanism of fragmental ions' with m/z 238.0110 formation.

Fragmented ion with m/z 165.0123 ($C_7H_7N_3S^+$) theoretically may exist during the break of the chemical bond between atom of sulfur and 1,2,4-triazole heterocyclic carbon (*fig. 4*).

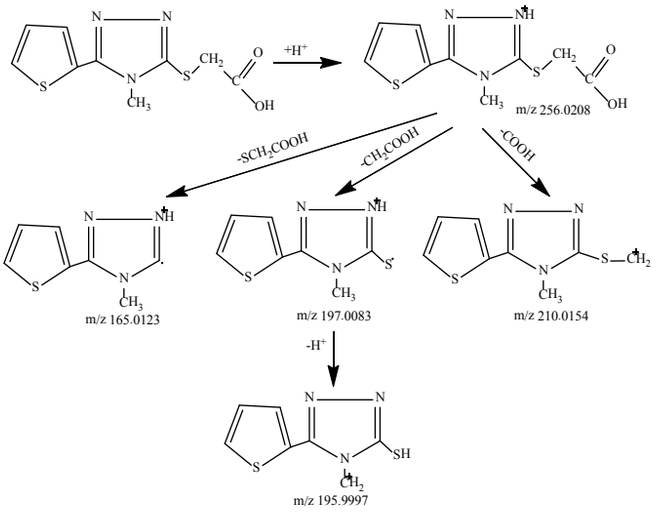

Fig. 4. Scheme of the fragmental ions' with m/z 210.0154, m/z 197.0083, m/z 195.9997 and m/z 165.0123 formation.

As a result of conducted research the most intensive peaks has been identified on the mass-spectrum received after the fragmentation of the protoned molecule 2-(4-methyl-5-(thiophene-2-yl)-4*H*-1,2,4-triazole-3-ylthio)acetic acid (MH^+).

Conclusions

1. The research of the sodium 2-(4-methyl-5-(thiophene-2-yl)-4*H*-1,2,4-triazole-3-ylthyo) acetate has been conducted for the first time.

2. As a result of experiment, mass-specters of the examined substance have been interpreted, typical ways of dissociation of the studied substance have been identified, and discussed reactions of the characteristic ions' formation have been offered.

3. It was found that at first the distruction of the molecule happens with the separation of the water molecule from the carboxyl group. Under the influence of the collision with gas the next may be separated the carboxyl group. During the further disintegration the bonds between the atom of sulfur and the acetous radical break or undergo the breaking of the covalent bond between sulfur and carbon of the 1,2,4-triazole.

References

1. Salionov, V. O., Pruhlo, Ye. S., Panasenko, O. I., & Knysh, Ye. H. (2013). Aktoprotektorna aktyvnist' pokhidnykh 2-(4-R-3-(tiofen-2-il)-4N-1,2,4-triazol-3-iltio)atsetatnykh kyslot [Actoprotective activity of derivatives of 2-(4-R-3-(thiophene-2-yl)-4H-1,2,4-triazol-3-iltio)acetic acid]. *Zaporozhskij medicinskij zhurnal,* 4(79), 51–53. [in Ukrainian].
2. Varynskyi, B. O. (2015). Optymizatsiia umov detektuvannia riadu 1,2,4-triazol-3-il-tioatsetatnykh kyslot ta yikh solei metodom VERKh-ESI-MS [Optimization of detecting a number of conditions 1,2,4-triazoles-3-yl-tioatsetatnyh acids and their salts by HPLC-ESI-MS]. *Odeskyi medychnyi zhurnal,* 4, 17–21. [in Ukrainian].
3. Varynskyi, B. O. (2015). Rozvytok VERKh-MS yak metodu otsiniuvannia chystoty i pidtverdzhennia molekuliarnoii masy novykh bioaktyvnykh rechovyn [Development of HPLC-MS as a method of evaluating the purity and confirm molecular mass of new bioactive compounds]. *Aktualni pytannia farmatsevtychnoi i medychnoi nauky ta praktyky,* 2, 85–88. [in Ukrainian].
4. Vulfson, N. S., Zaikin, V. G., & Mikaya, A. I. (1986) *Mass-spektry organicheskikh soedinenij* [*Mass spectra of organic compounds*]. Moscow. [in Russian].
5. Bushueva, I. V., Berezovskij, A. V., Knysh, Ye. H., & Panasenko, O. I. (2014). Zastosuvannia preparatu «Avesstym» dlia pidvyshchennia efektyvnosti vaktsynoprofilaktyky ta vplyv preparatu na rezystentnist' kurchat [Use of the drug «Avesstym» to improve vaccination and effects on the resistance of chickens]. *Science Rise,* 2014, 1(4), 94–97. [in Ukrainian].
6. Kazicina, L. A. (1979) *Primenenie UF-, IK-, YaMR- i MASS-spektroskopii v organicheskoj khimii* [*Application of UV, IR, NMR and mass spectrometry in organic chemistry*]. Moscow. [in Russian].
7. Kaplaushenko, A. H. (2013). Doslidzhennia zi stvorennia novoho oryhinal'noho vitchyznianoho likars'koho zasobu na osnovi 1,2,4-triazolu [Research on the creation of new original domestic drug based on 1,2,4-triazoles]. *Naukovyi zhurnal MOZ Ukrainy,* 2(3), 115–118. [in Ukrainian].
8. Iradyan, M. A., Iradyan, N. S., Grigoryan, R. T., & Panosyan, G. A. (2013). Metilovye e′firy i 5-(4N-1,2,4-triazol-3-ilsulfanil-metil)-furan-2-karbonovye kisloty. Sintez i mass-spektrometricheskoe issledovanie [Methyl ester and 5- (4H-1,2,4-triazol-3-ylsulfanyl-methyl)-furan-2-carboxylic acid. Synthesis and mass spectrometric investigation]. *Himicheskiy zhurnal Armenii,* 66(4), 636–647. [in Armenia].
9. Knysh, E. G., Parchenko, V. V., & Panasenko, A. I. (patentee) (2015). Veterinarnyj protivovirusnyj preparat [Pat. 2561674 Russia, IPC C 07 D 249/00. Veterinary antiviral drug. №2014102571/15. – declared 27.01.2015; published 27.08.2015. *Bulleten'*, 24. [in Russian].
10. Salionov, V. O., Panasenko, O. I., & Knysh, Ye. H. (2012). Syntez, fizyko-khimichni vlastyvosti 2-(4-R-5-(tiofen-2-il)-4N-1,2,4-triazol-3-iltio)atsetatnykh kyslot ta yikh solei [Synthesis, physico-chemical properties of 2-(4-R-5-(thiophene-2-yl) -4H-1,2,4-triazol-3-iltio)acetic acids and its salts]. *Ukrainskyi biofarmatsevtychnyi zhurnal*, 5–6, 114–117. [in Ukrainian].
11. Song, S. H., Jun, S. H., Park, U. K., Yoon, Y., Lee, J. H., Kim, J. Q., & Song, J. (2007) Simultaneous determination of first-line anti-tuberculosis drugs and their major metabolic ratios by liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom,* 21, 1331–1338.

Information about the authors:
Salionov V. A., Researcher, Teaching Assistant, Department of Inorganic Chemistry and Toxicology, Zaporizhzhia State Medical University, E-mail: salionov.88@mail.ru.
Varynskyi B. A., MD, PhD, Associate Professor, Department of Physical and Colloidal Chemistry, Zaporizhzhia State Medical University.
Parchenko V. V., MD, PhD, DSci, Associate Professor of the Department of Inorganic Chemistry and Toxicology, Zaporizhzhia State Medical University.
Відомості про авторів:
Саліонов В. О., здобувач, асистент каф. токсикологічної та неорганічної хімії, Запорізький державний медичний університет, E-mail: salionov.88@mail.ru.
Варинський Б. О., к. фарм. н., доцент каф. фізколоїдної хімії, Запорізький державний медичний університет.
Парченко В. В., д. фарм. н., доцент каф. токсикологічної та неорганічної хімії, Запорізький державний медичний університет.
Сведения об авторах:
Салионов В. А., соискатель, ассистент каф. токсикологической и неорганической химии, Запорожский государственный медицинский университет, E-mail: salionov.88@mail.ru.
Варинский Б. А., к. фарм. н., доцент каф. физколоидной химии, Запорожский государственный медицинский университет.
Парченко В. В., д. фарм. н., доцент каф. токсикологической и неорганической химии, Запорожский государственный медицинский университет.

Поступила в редакцию 05.10.2015 г.