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QUANTITATIVE DETERMINATION OF ETHACYSINE IN TABLETS BY SPECTROFLUOROMETRY AS ITS SULFONE

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The new method was elaborated for quantitative determination of ethacysine hydrochloride (the diethylamino analog of ethmozine) (ET) in the form of corresponding sulfonic derivative obtained with the use of potassium hydrogenperoxomonosulphate, through the spectrofluorometry (λ_{ex} = 264 nm/ λ_{em} = 380 nm). Linear concentration dependence was preserved in the concentrations interval (1-8)·10^{-6} mol/l ET, *lgI=97047c – 0.003 (r=0,999). LOQ =1.1·10^{-6} mol/l. It was showed that in the determination of ET in the tablets 50 mg (Olainfarm, Latvia) using the developed method,* RSD =1.7% (*accuracy,* δ =- 0.2%).

Keywords*: kinetic, potassium hydrogenperoxomonosulfate, ethacysine, spectrofluorometry, quantitative determination*

Introduction. Ethacysine (*sin.* Aethacizin; Etacizin; Ethacizin; Ethacyzin; EZ-55; NIK-244) – ethyl N-[10-[3-(diethylamino) propanoyl]phenothiazin-2-yl]carbamate hydrochloride (ET) – belongs to 10-acyl derivatives of phenothiazine (the diethylamino analog of ethmozine) and is used in medicine as the antiarrhythmic agent [1] (fig. 1). It is produced in the form of 2.5% solution for injections in 2 ml ampoules, and also 0.05 g tablets (manufactured by Olainfarm, Latvia).

Despite the wide application of ET in medical practice, analytical method of quantitative determination of this pharmaceutical preparation has not been investigated enough.

For quantitative determination of ET in medical preparations and biological fluids the BEPX method was suggested [2,3] of direct ultraviolet spectrophotometry [4], photoelectrocolorymetry in the form of oxydative-hydrolitic decomposition product in the sulfuric acid environment [5]. For the purpose of detecting the falsified medicines (identity clarification) the methods TLC, UV, and IR-spectroscopy were suggested [6].

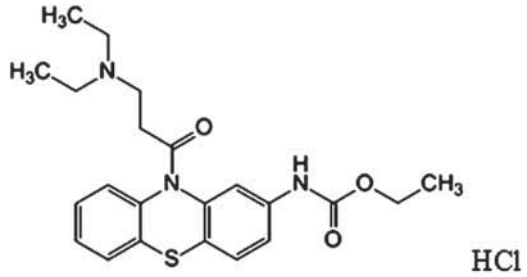

Fig. 1. Ethacysine hydrochloride structure

Besides, in the literature a number of original articles were found describing the highly-sensitive spectrofluorometric methods of identification and quantitative determination of the phenothiazine derivatives in different medicines [7-9]. However, the ET fluorescent characteristics have not been studied before, and there appeared to be no methods.

The aim of this article is to provide a detailed investigation of the kinetics of ET oxidation with the potassium hydrogenperoxomonosulfate, and fluorescence spectrums of ET and its oxidation products for development of the unified highly-sensitive and selective method of quantitative ET determination in the pharmaceutical preparations.

Experimental section

Instruments, matrials, reagents and methods

Ethacysine hydrochloride, substance-powder**,** manufactured by FSUC State Research Center of Organic Products and Colorants (NIOPIK, Russia) complying with the ND 42-8072-97.

Ethacysine tablets 0.05 g produced by AS Olainfarm, Latvia (ser. 280615). Film-coated tablets: tablet core: active substance: Ethacysine hydrochloride (ethyl N- [10- [3- (diethylamino) propanoyl] phenothiazin-2-yl] carbamate hydrochloride) 50 mg of additive agents: potato starch – 9.57 mg; sucrose - 19.3 mg; microcrystalline methylcellulose – 0.33 mg; calcium stearate – 0.8 mg shell: sucrose – 37.695 mg; povidone - 0.753 mg; quinoline yellow dye (E104) – 0.025 mg; dye "sunset" yellow FCF (E110) – 0.003 mg; calcium carbonate - 6.308 mg; magnesium hydroxycarbonate main – 3.678 mg; titanium dioxide (E171) - 0.665 mg; silica dioxide – 0.827 mg; wax Carnuba Wax – 0.046 mg.

Oxone®, monopersulfate ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) (SIGMA-ALDRICH), CAS: 70693-62-8 (further as *oxone*), Active oxygen (AO) 4.5 % w/w.

For preparation of $4 \cdot 10^{-2}$ mol/l of the initial solution of *potassium* hydrogenperoxomonosulfate ($KHSO_5$) the sample weight 0.615 g oxone was diluted in 50 ml double-distilled water. Solutions were kept for a week at the room temperature. The solution with the concentration of $2.2 \cdot 10^{-3}$ mol/l was received through the corresponding dilution of double-distilled water.

The standard ET solutions were prepared at the exact sample weights of preparation substance on the double-distilled water. The working standard solutions of ET were prepared out of the initial solutions through the corresponding dilution with double-distilled water. All solutions were kept at the room temperature in the dark cool place.

The absorption and fluorescence spectrums were recorded at the temperature of 20ºC on the fluorescent spectrophotometer MPF-4 «Hitachi», equipped with the specialized MPF computer (612-0655). The gauge and recording of the fluorescence

spectrums of the researched ET oxidized derivatives were conducted at least 5 times, averaged and deducted the averaged specter of base solution (without the determined derivative: potassium hydrogenperoxosulphate taking into account the oxidation stoichiometry).

Oxone solution standardization procedure. The composition of active oxygen in the oxone samples and concentration of potassium hydrogenperoxosulphate solutions were determined using the iodometric titration method: precisely weighted amount of oxone is diluted in 10-15 ml of double-distilled water, acidified with 1-2 ml 0.1 M dipping acid solution, added 1 ml potassium iodide solution 5% and free iodine was titrated with 0.02 M of standard sodium thiosulphate solution using 10 ml microburette. The amount of standard test reagent was measured with the accuracy of ±0.01 ml.

Standard sodium thiosulphate solution was prepared of the standard titre fixanal ampoule on the double-distilled water. Titrated 0.02 M thiosulphate solution was prepared through the corresponding dilution of the initial solution in the newly boiled double-distilled water with the addition of chemically pure sodium carbonate [10].

The solutions pH were prepared using the electrometric compensation method on the laboratory ion-meter И-130 with the glass electrode ЭСЛ-43-07 together with «SSCE» (sat. Silver/Silver Chloride Electrode).

The necessary environment acidity was maintained using the buffer solutions prepared on KH_2PO_4 and K_2HPO_4 according to Green [11]. The S-oxidation kinetics of phenothiazine derivatives was studied using the methods of samples selection according to the discharge of potassium hydrogenperoxosulphate (iodometric titration of oxidant residue).

Methodology of the reaction kinetics studying using the iodometric titration method. Into 100ml measuring flask 20-30 ml buffer solution, 20. 0 ml $1\cdot10^{-2}$ mol/l potassium hydrogen peroxomonosulphate and 5.0 ml $1\cdot10^{-2}$ mol/l ET solution were sequentially poured (the stopwatch started), shaking the solution in the flask immediately the volume to was brought to the mark, corked and thoroughly mixed turning the flask. Then after some time using the 10 ml pipette reaction mixture was selected and while mixing poured into the conic flask with 1 ml 5% potassium iodide and 5 ml 0.1 mol/l dipping acid solution. The released iodine was titrated with 0.02 mol/l solution of sodium thiosulphate measuring the volume with an accuracy of ±0,01 ml.

Spectrums of fluorescence of ET solutions of concentration (solution pH) for the maximum excitation band (λ_{ex}, 264 nm) position of maximum emission band, λ_{em}, 392 nm: ETO (ET sulfoxide) $1\cdot10^{-5}$ mol/l (pH 5.6; 0.02 mol/l KH_2PO_4 and K_2HPO_4) (264) 380. ETO_2 (ET sulfone) $1\cdot10^{-5}$ M (9.2, 0.02 mol/l K_2HPO_4) (264) 380 (fig. 2 and 3).

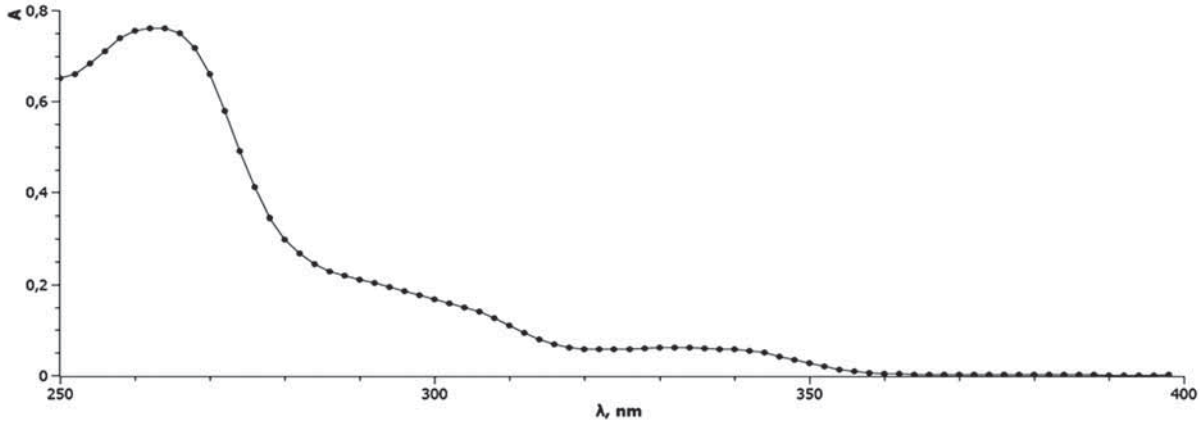

Fig. 2. Electronic absorption spectrum ETO_2 c(ETO_2)=2.2·10^{-5} mol/l; pH=**9.2**

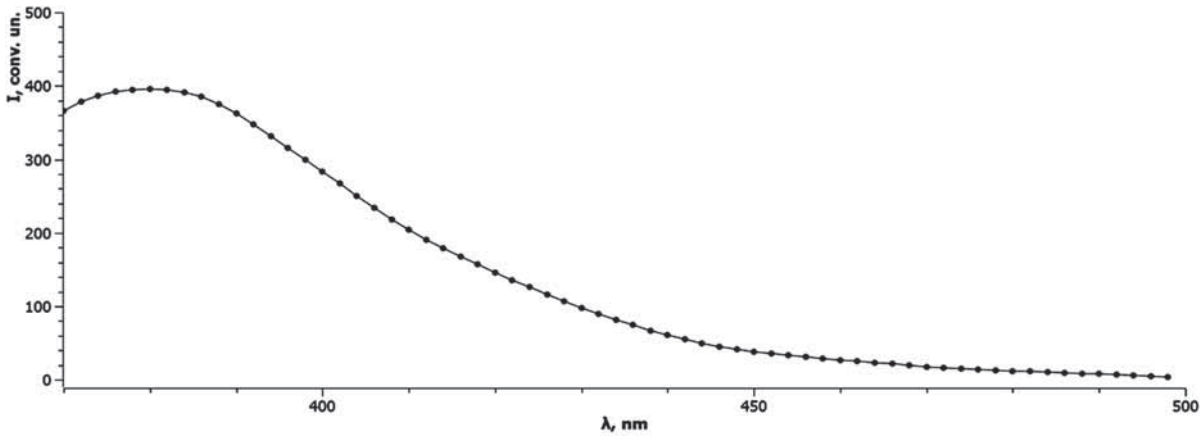

Fig. 3. Fluorescence spectrum of ETO_2 c(ETO_2)=2.2·10^{-5} mol/l; pH=**9.2**

Kinetics of ETO oxidation reaction was also studied spectrofluorimetrically according to the formed oxidation product (ETO_2) at 380 nm, the cell thickness l = 1 sm, for the solutions mixing the Budarin's reactor was used [12], the time was recorded using the stopwatch from the moment of solutions mixing. Before draining the solutions were thermostated in the thermostat UTU-2 (Zeamit, Horizont Krakow-Poland) at 20 ±0.5° C. The reactions constants ($k_{еф}$) were found by the slope ratio of the initial sections of kinetic time curves lnI_{fl}.

Results and discussion

The kinetics studying results showed that at c($KHSO_5$)=$1.77\cdot10^{-3}$ mol/l; c(ET)=$4.7\cdot10^{-4}$ mol/l ET oxidation takes place quantitatively and stoichiometrically with the formation of corresponding sulfoxide of ET (ETO) and sulfone of ET (ETO_2) etacisin derivative: in acid medium (pH 5.6-6.5) per 1 mol ET 1 mol $KHSO_5$ (formation of ETO) is spent, and in the alkaline medium (pH 8.5-9.2) – 2 mol $KHSO_5$ (ETO_2 formation). Stoichiometric ETO formation is achieved practically immediately (observation period 1min); ETO_2 is quantitatively formed during the period not exceeding 15 min (fig. 4).

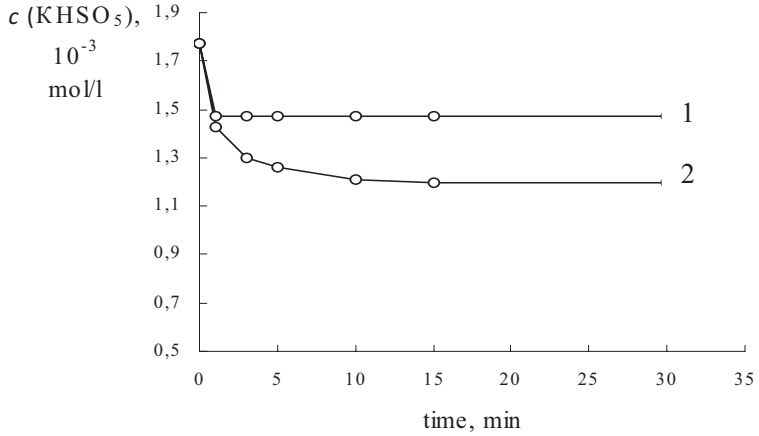

Fig. 4. Kinetic curves of ET **oxidation using potassium hydrogenperoxomonosulphate** c($KHSO_5$)=**1.77·10^{-3}** моль/л; c**(ET)=4.7·10^{-4}** моль/л; pH **:** ***1*** **– 5.6;** ***2*** **– 8.5-9.2.**

The fig. 5 provides the general scheme of reaction of ET S-oxidation using potassium hydrogenperoxomonosulphate.

$$R_2S + KHSO_5 \rightarrow R_2SO + KHSO_4 \ (pH<7)$$
$$R_2SO + KHSO_5 \rightarrow R_2SO_2 + KHSO_4 \ (pH\geq 7)$$

Fig. 5. Scheme of ET oxidation using potassium hydrogenperoxomonosulphate

It was determined that reaction of ETO oxidation into ETO_2 is biomolecular, first-order with two reagents. Under the conditions of pseudo-first reaction behavior order ($KHSO_5$ surplus) the k_{ef} were calculated. The ET oxidation reaction equation was derived which looks as follows:

- d[ET]/dt = k_1[ETO], d[ETO]/dt = k_1[ET] – k_2[ETO], d[ETO_2]/dt = -k_2[ETO], $k_1 >> k_2$.

The quantitative sulfone of ET formation was achieved for 15 min in the presence of oxidant surplus at pH≥8,5. Under the comparative conditions the fluorescence of ET sulfonic derivative is next stronger than that of unoxidized ET or partially oxidized derivative (ET sulfoxide). The highest fluorescence was observed in the alkali water solution with pH 9.2. Based on the results received the relatively simple and quite sensitive method was developed of spectrofluorimetric ET determination in the coated 0.05 g tablets (manufactured by «Olainfarm», Latvia). The method was based on the formation of intensely fluorescent S-oxidation product, formed at the interaction of ET with potassium hydrogenperoxomonosulfate in the alkali medium (pH 9.2).

Into the measuring flask 25 ml of thoroughly filtered through the paper filter (blue bond) analyzed pills solution (or working standard sample) ET was poured, the oxone(surplus) solution was added as well as the buffer mixture solution, and, thus the volume was brought up to the mark with double-distilled water and thoroughly mixed. After 15 min of storage the fluorescence of the received oxidation product was measured (λ_{ex} = 264 nm/ λ_{em} = 380 nm). The preparation composition was determined using the standard method, taking into account the dilution.

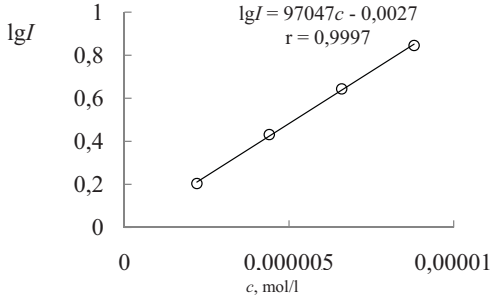

Fig. 6. lg I_{fl} dependence from concentration of ETO_2 (pH **9.2)**

Linear concentration dependence was preserved within the concentrations range (1-8)·10^{-6} mol/l ET, lg*I*=(97.0±7.9)·10^3*c*, where c in mol/l (r=0.999) (Fig. 6). Using the method of «introduced (μ)- found ()» the analysis results correctness was verified, δ < RSD, where δ= (*n*=5, P=0.95). It was showed that when determining the ET in the tablets 50 mg manufactured by Olainfarm using the researched method RSD =1.7% (δ =- 0.2%, as compared with the certificate data).LOQ =1.1·10^{-6} mol/l. The content of the active pharmaceutical ingredient (API) was 50.3 mg (at admission 47.5- 52.5 mg) to one tablet.

Conclusions

The kinetics was studied of the reaction of ethacysine S-oxidation using the potassium hydrogenperoxomonosulphate in the acid and alkali medium under the conditions of oxidant surplus. The oxidation products identification was conducted.

The study was conducted of the simple, selective, and sensitive method of the quantitative ethacysine determination in the form of corresponding sulfonic derivative (ethacysine sulfone) using the spectrofluorometry method in the tablets 0.05 g.

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ОРГАНИЧЕСКОЕ ВЕЩЕСТВО В МЕЖЗВЕЗДНОЙ СРЕДЕ

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В составе межзвездных облаков установлено наличие 200 различных молекул и частиц, большая часть которых имеет органическую природу и является составной частью нефти. Показано, что в состав межзвездной среды входят межзвёздный газ, пыль, межзвёздные магнитные поля, космические лучи, а также тёмная материя. Межзвездная пыль содержит водяной лед, силикаты, графит, оливин, оксиды и сульфиды металлов и покрыта сверху оболочкой из намерзших газов. Температура пыли в межзвездном пространстве около 10 – 20K. Показано, что при низких температурах органические вещества образуются на поверхности пылинки в десятки раз быстрее, чем при комнатной температуре. Обсуждается, что причиной ускорения скорости при низких температурах для газофазных реакций является квантовое туннелирование, а для твердофазных реакций - механизм типа явлений бегущей волны.

Ключевые слова: *органическое вещество, космос, межзвездная среда, межзвездная пыль, криохимия, механизм реакции, эффект туннелирования, явление бегущей волны.*

The composition of interstellar clouds revealed the presence of 200 different molecules and particles, most of which is organic in nature and is an integral part of oil. It is shown that the composition of the interstellar medium consists of interstellar gas, dust and interstellar magnetic fields, cosmic rays, and dark matter. Interstellar dust contains aqueous ice, silicates, graphite, olivine, oxides and sulfides of metals and topped shell of the frozen gas. Temperature of dust in interstellar space is around 10 - 20K. It is demonstrated that at low temperatures the organic matter are formed on the surface of dust particles in the tens times faster than at room temperature. It is discussed the cause of the acceleration rates at low temperatures for gas phase reactions is the quantum tunneling, while for solid-state reactions -mechanism such as a traveling wave phenomena.

Keywords: *organic matter, space, interstellar medium, interstellar dust, cryochemistry, reaction mechanism, tunneling effect, the phenomenon of the traveling wave.*

Межзвёздная среда - вещество и поля, заполняющие межзвёздное пространство внутри галактик. В состав межзвездной среды входят межзвёздный газ, пыль (1 % от массы газа), межзвёздные магнитные поля, космические лучи, а также тёмная материя. Химический состав межзвёздной среды - продукт первичного нуклеосинтеза и ядерного синтеза в звёздах. А в конце жизни звезды с неё сбрасывается оболочка, обогащая межзвёздную среду продуктами ядерного синтеза. Пространственное распределение межзвёздной среды нетривиально. Помимо общегалактических структур, таких как перемычка (бар) и спиральные рукава галактик, есть и отдельные холодные и тёплые облака, окружённые более горячим газом. Основная особенность межзвёздной среды - её крайне низкая плотность - в среднем 1000 атомов в кубическом сантиметре.

Химический состав Вселенной зависит от многих факторов, в том числе и от температуры. По мере повышения температуры состав частиц, существующих в атмосфере звезды, упрощается. Так, спектральный анализ звезд с температурой 10 000-50 000°C показывает в их атмосферах линии ионизированных водорода и гелия и ионы металлов. В атмосферах звезд с температурой 5000°C обнаруживаются уже радикалы, а в атмосферах звезд с температурой 3800°C – даже молекулы оксидов. В спектрах самых горячих звезд преобладают линии водорода и гелия, но по мере понижения температуры появляются линии других элементов и даже линии соединений. Это еще простые соединения: оксиды циркония, титана, а также радикалы CH, OH, NH, CH_2, C_2, C_3, CaH и др. Наружные слои звезд состоят главным образом из водорода. В среднем на 10 000 атомов водорода приходится около 1000 атомов гелия, 5 атомов кислорода и менее 1 атома других элементов [1].

В природе самая низкая температура была зарегистрирована в туманности Бумеранг. Эта туманность расширяется и выбрасывает охлажденный газ со скоростью 500 000 км/ч. За счет огромной скорости выброса молекулы газа охладились до 2 К. **Для сравнения.** Обычно, в открытом космосе температура опускается до 0К. Самая низкая естественная температура на Земле 183.7 К в Антарктиде [2]. А самая низкая температура в Солнечной системе, 38К на поверхности Тритона (спутник Нептуна).

В этом обзоре основное внимание уделено на образование органических веществ в условиях космоса. Исходя из этого, целью этой статьи является разъяснить, сколько и какие классы органических соединений идентифицированы в космосе и особенности химических реакций их образования в космических условиях.