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 analogue of ethmozine). It 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 ВЕРХ method was suggested [2,3] - direct ultraviolet spectrophotometry [4], photoelectrocolorymetry in the form of oxidative-hydrolitic decomposition product in the sulphuric acid environment [5]. For the purpose of detecting the falsified medicines (identity clarification) the methods of TLC, UV, and IR-spectroscopy were suggested [6].

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 paper is to provide a detailed investigation of the kinetics of ET oxidation with the potassium hydrogenperoxomonosulphate, and fluorescence spectrums of ET and its oxidation products in order to develop the unified highly-sensitive and selective method of quantitative ET determination in the pharmaceutical preparations.

Experimental section

Instruments, materials, reagents and methods

Ethacysine hydrochloride, substance-powder, manufactured by FSUC State Research Centre 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, with 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 (2KHSO₅·KHSO₄·K₂SO₄) (SIGMA-ALDRICH), CAS: 70693-62-8 (in further – oxone), Active oxygen (AO) 4.5 % w/w.

For preparation of 4·10⁻² mol/l of the initial solution of potassium hydroperxomonosulphate, ET was taken in different concentrations in the range of (1-8)·10⁻⁶ mol/l ET.

The new method was elaborated for quantitative determination of ethacysine hydrochloride (the diethylamino analogue of ethmozine) (ET) in the form of the corresponding sulfonic derivative obtained with the use of potassium hydrogenperoxomonosulphate, through the spectrofluorometry (λex = 264 nm/ λem = 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 shown that in the determination of ET in the tablets of 50 mg (Olainfarm, Latvia) using the developed method, RSD =1.7% (accuracy, δ = 0.2%).

Keywords: kinetic, potassium hydrogenperoxomonosulphate, ethacysine, spectrofluorometry, quantitative determination.

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hydrogenperoxomonosulphate (КНСО₅) the sample weight 0.615 g of 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 2.2·10⁻³ mol/l was received through the corresponding dilution of 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 20°C 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 spectre 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 of 0.1 M dipping acid solution, added 1 ml of potassium iodide solution 5%, and free iodine was titrated with 0.02 M of standard sodium thiosulphate solution using the 10 ml microburette. The amount of standard test reagent was measured with the accuracy of ±0.01 ml.

Standard sodium thiosulphate solution was prepared using 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 pH solutions were prepared using the electrometric compensation method on the laboratory ion-meter "I-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 КН₂РО₄ and К₂НРО₄ 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 the oxidant residue).

Studying the methodology of the reaction kinetics using the iodometric titration method. Into 100 ml measuring flask 20-30 ml buffer solution, 20.0 ml of 1·10⁻³ mol/l potassium hydrogenperoxomonosulphate and 5.0 ml of 1·10⁻⁴ mol/l ET solution were sequentially poured (the stopwatch started); shaking the solution in the flask immediately the volume was brought to the mark; corked
and thoroughly mixed by turning the flask. Then after some time using the 10 ml pipette the reaction mixture was taken and while mixed poured into the conic flask with 1 ml of 5% potassium iodide and 5 ml of 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.

Spectra of fluorescence of ET solutions of concentration (pH solution) for the maximum excitation band (λex: 264 nm), position of maximum emission band, λem: 392 nm: ETO (ET sulfoxide) 1·10⁻⁵ mol/l (pH 5.6; 0.02 mol/l KH₂PO₄ and K₂HPO₄) (264) 380. ETO2 (ET sulfone) 1·10⁻⁴ M (9.2, 0.02 mol/l KH₂PO₄) (264) 380 (fig. 2 and 3).

Kinetics of ETO oxidation reaction was also studied spectrophotometrically according to the formed oxidation product (ETO₂) 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°С. The reactions were thermostated in the thermostat UTU-2 (Zeamit, Horizont Krakow-Poland) at 20±0.5°С. The reactions were conducted.

The kinetics was studied of the ET S-oxidation using potassium hydrogenperoxomonosulphate (K₂H₂O₂S₄) in the acid and alkali medium under the conditions of oxidant surplus. The preparation composition was determined using the standard method, taking into account the dilution.

Linear concentration dependence was preserved within the concentrations range of (1-8)·10⁻⁶ mol/l ET, lg I = (97.0±7.9)·10⁻⁷·c, where c in mol/l (r=0.999) (Fig. 6). Using the method of "introduced (μ) – found (兮)" the analysis results correctness was verified, δ < RSD, where δ = \( \frac{(兮-μ)}{\mu} \) (n=5, P=0.95). It was shown that when determining the ET in tablets (50 mg) manufactured by Olainfarm using the researched method RSD = 1.7% (δ = – 0.2%, as compared to the certificate data). LOQ = 1·10⁻⁶ mol/l. The content of the active pharmaceutical ingredient (API) was 50.3 mg (at admission 47.5-52.5 mg) in one tablet.

**Conclusions**

1. 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.

**Results and discussion**

The results showed that at c(KHSO₅) = 1.77·10⁻¹ mol/l; c(ET) = 4.7·10⁻⁶ mol/l ET oxidation takes place quantitatively and stoichiometrically with the formation of the corresponding sulfoxide of ET (ETO) and sulfone of ET (ETO₂) ethacysine derivative: in acid medium (pH 5.6-6.5) per 1 mol of ET 1 mol of KHSO₅ (formation of ETO) is spent, and in the alkaline medium (pH 8.5-9.2) – 2 mol of KHSO₅ (ETO₂ formation). Stoichiometric ETO formation is achieved practically immediately (observation period 1 min); ETO₂ is quantitatively formed during the period not exceeding 15 min (fig. 4).

**Fig. 5. Scheme of ET oxidation using potassium hydrogenperoxomonosulphate**

**Fig. 6. lg Iₓ dependence on concentration of ETO₂ (pH 9.2)**
2. The study was conducted in relation to 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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