U.D.C. 615.074: 615.222: 543.426: 543.431: 543.062

QUANTITATIVE DETERMINATION OF ETHACYSINE IN TABLETS BY SPECTROFLUOROMETRY AS ITS SULFONE

M.Ye. Blazheyevskiy[1], Doctor of Chemistry, Full Professor
A.O. Doroshenko[2], Droctor of Chemistry, Full Professor
V.O. Yevtukhov[3], Student
National University of Pharmacy, Ukraine[1]
V.N. Karazin Kharkiv National University, Ukraine[2,3]

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 (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 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.

Conference participants,
National championship in scientific analytics,
Open European and Asian research analytics championship

http://dx.doi.org/10.18007/gisap:pmc.v0i8.1552

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).

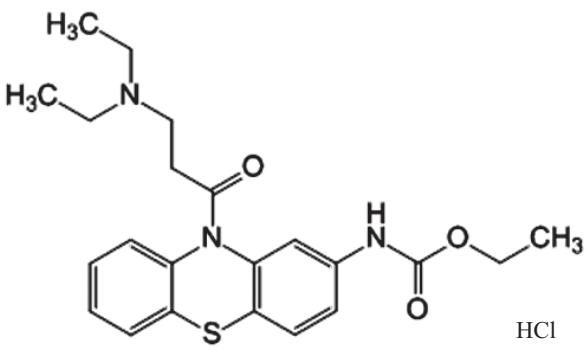

Fig. 1. Ethacysine hydrochloride structure

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] - direct ultraviolet spectrophotometry [4], photoelectrocolorymetry in the form of oxydative-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, matrials, 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 ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) (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*

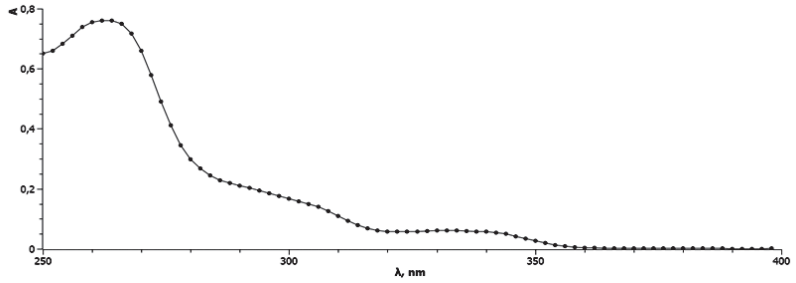

Fig. 2. Electronic absorption spectrum of ETO_2 $c(ETO_2)=2.2\cdot10^{-5}$ mol/l; pH=9.2

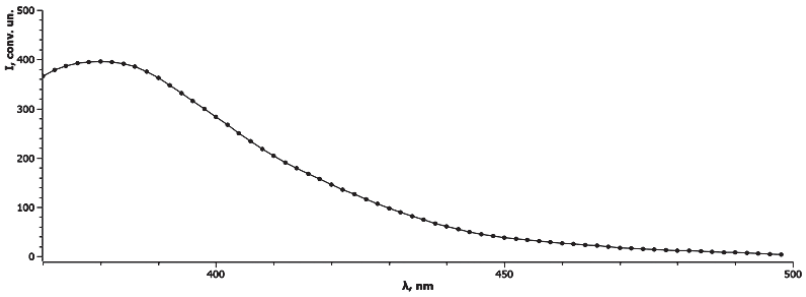

Fig. 3. Fluorescence spectrum of ETO_2 $c(ETO_2)=2.2\cdot10^{-5}$ mol/l; pH=9.2

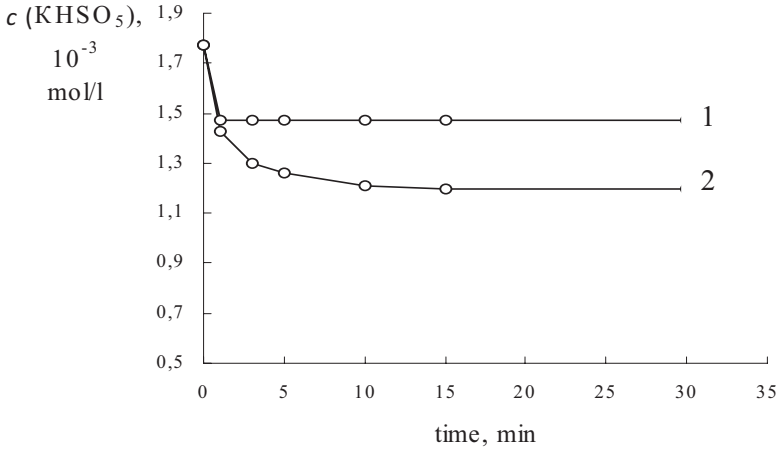

Fig. 4. Kinetic curves of ET oxidation using potassium hydrogenperoxomonosulphate $c(KHSO_5)=1.77\cdot10^{-3}$ моль/л; $c(ET)=4.7\cdot10^{-4}$ моль/л; pH : *1* – 5.6; *2* – 8.5-9.2.

hydrogenperoxomonosulphate ($KHSO_5$) 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 $2.2\cdot10^{-3}$ 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 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 "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 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 $1\cdot10^{-2}$ mol/l potassium hydrogen peroxomonosulphate and 5.0 ml of $1\cdot10^{-2}$ 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 mixing 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.

Spectrums of fluorescence of ET solutions of concentration (pH solution) 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. ETO2 (ET sulfone) $1\cdot10^{-5}$ M (9.2, 0.02 mol/l K_2HPO_4) (264) 380 (fig. 2 and 3).

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 ($k_{eф}$) were found by the slope ratio of the initial sections of kinetic time curves $\ln I_{fl}$.

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 the corresponding sulfoxide of ET (ETO) and sulfone of ET (ETO_2) ethacysine derivative: in acid medium (pH 5.6-6.5) per 1 mol of ET 1 mol of $KHSO_5$ (formation of ETO) is spent, and in the alkaline medium (pH 8.5-9.2) – 2 mol of $KHSO_5$ (ETO_2 formation). Stoichiometric ETO formation is achieved practically immediately (observation period 1 min); ETO_2 is quantitatively formed during the period not exceeding 15 min (fig. 4).

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

It was determined that reaction of

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

$$R_2SO + KHSO_5 \rightarrow R_2SO_2 + KHSO_4 \,(pH\geq7)$$

Fig. 5. Scheme of ET oxidation using potassium hydrogenperoxomonosulphate

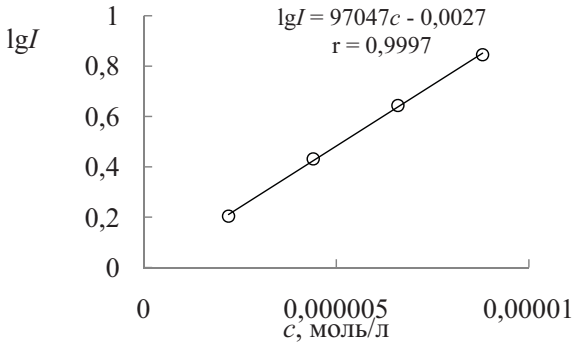

Fig. 6. lg I_{fl} dependence on concentration of ETO_2 (pH 9.2)

ETO oxidation into ETO_2 is biomolecular, first-order with two reagents. Under the conditions of pseudo-first reaction behaviour order ($KHSO_5$ surplus) the kef 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 - 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) analysed pills solution (or working standard sample) of 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.

Linear concentration dependence was preserved within the concentrations range of $(1\text{-}8)\cdot10^{-6}$ mol/l ET, $lgI=(97.0\pm7.9)\cdot10^3 c$, where c in mol/l (r=0.999) (Fig. 6). Using the method of "introduced (μ) – found ($\bar{x}$)» the analysis results correctness was verified, δ < RSD, where $\delta = \frac{(x-\mu)100}{\mu}$.

(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 toh the certificate data). LOQ =$1.1\cdot10^{-6}$ 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.

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).

References:

1. Kovalenko V.N. Compendium 2014 – medicine drugs. K. Morion. 2014 – 2448 p. (Russian)

2. Prokof'eva V.I., Chernova S.V., Kashtanova V., Shavratskii V.Kh., Gneushev E.I. Use of high-performance liquid chromatography in the evaluation of purity of ethacizine., Pharm. Chem. J. – 1990., Vol. 24, No. 4., pp. 306-307.

3. Beloborodov V.L., Zalesskaya M.A., Tyukavkina N.A. Quantitative Determination of Metacizine Components in Biological Fluids., Khimiko-Farmatsevticheskii Zhurnal [Pharmaceutical Chemistry Journal]. – 2000., Vol. 34, No. 12., pp. 41-44. (Russian)

4. Kubrak Z.V., Popova V.I. Quantitative determination of ethacizine by UV spectrophotometry. Farmatsevtychnyi Zhurnal (Kiev). – 1992, No. 1., pp. 79-80. (Ukrainian)

5. Kubrak Z.V., Byeikin S.H. Ethacizine determination in biological fluids., Zhurnal (Kiev). – 1989., No. 2., pp. 69-70. (Ukrainian)

6. Kuvyrchenkova I.S. Methods of analysis of phenothiazine derivatives., Pharmacy. – 2006., No. 6., pp. 18–21. (Russian)

7. Mohamed F.A. Spectro-fluorimetric determination of chlorpromazine hydrochloride and thioridazine hydrochloride., Anal. Lett. – 1995., Vol. 28, No. 14., pp. 2491-2501. https://doi.org/10.1080/00032719508004030

8. Yang G.J., Qu X.L., Shen M., Qu Q.S., Wang C.Y., Zhu A.P., Hu X.Y. Trace measurement of phenothiazine drugs in tablets by micellar-enhanced fluorophotometric method., Fluoresc. – 2007., Vol. 17, No. 2., pp. 119-126. https://doi.org/10.1007/s10895-007-0165-5

9. Shlyusar O.I., Blazheyevs'kyy M.Ye., Aleksandrova D.I. Kil'kisne vyznachennya tryfluoperazyn v likars'kykh preparatakh metodom spektrofluorymetriyi u vyhlyadi S-oksydu [Quantitative determination of trifluoperazine in pharmaceuticals with the help of spectrofluorometry in the C-Oxide type]., Medychna khimiya [Medical Chemistry]. – 2012., T. 14, No. 2(51)., pp. 39-43 (Ukrainian)

10. Suslennikova V.M., Kiseleva E.K. Rukovodstvo po prigotovleniyu titrovannykh rastvorov [Guide for preparing the volumetric solutions]. – Leningrad., Khimiya [Chemistry], 1978. – 184 p. (Russian)

11. Spravochnik khimika-analitika., A.I. Lazarev, I.P. Kharlamov, P.Ya. Yakovlev, Ye.F. Yakovlev – Moskow., Metallurgiya, 1976. – 184 p. (Russian)

12. Yatsimirskiy K.B. Kineticheskie metody analiza [Kinetic methods of analysis]., Khimiya Publ., 1967. – 200 p. (Russian)

Information about authors:

1. Vladyslav Ievtukhov – Student; V.N. Karazin Kharkiv National University; address: Ukraine, Kharkiv city; e-mail: vlad.e.06.95@gmail.com

2. Mykola Blazheyevskiy – Droctor of Chemistry, Full Professor, National University of Pharmacy; address: Ukraine, Kharkiv city; e-mail: blazejowski@ukr.net

3. Andrey Doroshenko – Droctor of Chemistry, Full Professor, V.N. Karazin Kharkiv National University; address: Ukraine, Kharkiv city; e-mail: vlad.e.06.95@gmail.com