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Quantitative determination of ampicillin and oxacillin in the "Ampiox" preparation using potassium hydrogenperoxomonosulphate

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ABSTRACT

Kinetics of the reactions of ampicillin and oxacillin peroxyacidic oxidation and perhydroliysis with potassium peroxomonosulphate in the alkaline medium by product light adsorption was studied. The procedure and the possibility of quantitative determination of ampicillin and oxacillin in the Ampiox preparation by the kinetic-spectrophotometric and iodometric methods were developed. RSD ≤ 1.5 % (δ = 1,.4..– 0.5 %).

Keywords: spectrophotometry, "Ampiox", potassium hydrogenperoxomonosulphate, perhydrolysis

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INTRODUCTION

Among the peroral forms of the produced domestically medicinal products of the penicillin series, the combined preparation "Ampiox", whose composition includes the semisynthetic penicillins of the first generation – ampicillin trihydrate and oxacillin sodium salt in the ratio 1:1 – has a special place. It is manufactured as gelatine capsules in the dosage of 0.25 g of the preparation [4]. The preparation is of the broad antibacterial spectrum, which covers the spectra of ampicillin and oxacillin. The content of penicillins' sum in the preparation can be determined by the method of iodometric titration, which presupposes analysing the alkaline hydrolysis of the tested penicillins at the first stage [2]. In parallel and under the same conditions, the standard sample of the preparation is to be analysed. The various modifications of this technique are described. Its shortcoming is the dependence of the titre value upon the temperature.

The spectrophotometric methods of the quantitative determination of ampicillin and oxacillin in "Ampiox" are being narrowed down to the determining the products of their hydrolytic decomposition, they are long-lasting and need heating [3, 6, 8, 12].

The State Pharmacopoeia of Ukraine recommends to monitor the content thereof in the "Ampiox" preparation by means of the method of the high-performance liquid chromatography (HPLC), which makes it possible to simultaneously determine quantitatively the both components and to identify them [5, 7, 9].

The relatively fast and easy-to-perform kinetic analysis techniques, based on the perhydrolysis reactions of the β-lactam ring with recording the generated with time products by spectrophotometry method [1, 10] are of interest.

Our work deals with the development of the new differential kinetic spectrophotometric method of the quantitative determination of ampicillin in the combined "Ampiox" preparation on the base of the product of two conjugated reactions – peroxyacid oxidation and perhydrolysis in the alkaline medium. The content of oxacillin in the preparation is to be found based on the difference between the total content of penicillins and the content of ampicillin, obtained by the kinetic method.

A simple and enough selective kinetic-spectrophotometric procedure for quantitative determination of ampicillin and oxacillin in the combined two-componential "Ampiox" preparation (0.125 g of ampicillin trihydrate and 0.125 g of sodium oxacillin) was developed. It is based on the oxidation of both antibiotics by potassium hydrogenperoxomonosulphate during 1 min to corresponding S-oxides and further basic hydrolysis at room temperature. The rate of reaction was determined at 302 nm and characterized by the angle tangent of the linear part of the kinetic curve A against time, min. For both antibiotics under study graduation dependences had straight linear dependence in the concentration range 1–50 μg mL^{-1}. Combination of the results of iodometric determination (by the potassium hydrogenperoxomonosulphate excess reverse titration in the reaction of S-oxidation in independent experiment) of the summary components content in the mixture with the results of kinetic determination of fast reacting component (ampicillin) allows to calculate the penicillin's' content in binary mixture. LOD for ampicillin and oxacillin were 1.0 μg mL^{-1} and 2.0 μg mL^{-1} respectively. Accuracy and precision of the results was approved by the method „taken-found" analyzing the prepared mixture (model of "Ampiox" preparation) with the precise components content *RSD* ≤1.9 % (δ=+0.8…–0.75%). The obtained results of the average components content in medicinal preparation of factory production show excellent agreement with pharmacopoeia analysis results from the quality certificate

The developed procedure of penicillin's kinetic determination has a lot of advantages: allows to determine significantly less concentration than while pharmacopoeia titrimetric method; can be applied to the same concentration range as in photometric hydrolysis products, but does not require long-lasting heating of the reaction mixture, simpler than chromatography procedures and quicker.

EXPERIMENTAL SECTION

The "Ampiox" preparation in the capsules of 0.250 g of series 291110, manufactured by the "PHARMACEUTICAL COMPANY "ZDOROVIE" LTD., Kharkiv, Ukraine, was applied for the studies. As an oxidizing agent, the potassium triple salt of peroxymonosulfuric acid, $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$, (Syn. "Oxone"), (ACROS ORGANICS), was used. Its active agent is potassium hydrogen peroxomonosulphate, $KHSO_5$. The choice of the reagent is to be explained by its accessibility, satisfactory solubility in water, relatively high oxidation capability (E^o = 1.81 V) as well as by sufficient tolerance during storage and application. To ensure the needed solubility of the ampicillin trihydrate, N,N-dimethylformamide (DMFA) chemically pure was used.

The sodium thiosulphate titrated solution $c(Na_2S_2O_3 \cdot 5H2O, f=1)$ = 0.1 mol L^{-1} is produced from the fixanal. The more dissolved solutions were obtained through the relevant dilution of the original solution by distilled water.

1% solution of potassium iodide. 1.0 g of potassium iodide is to be dissolved in the just boiled as well as chilled distilled water; the volume of the solution is to be brought up to 100 mL.

The sulphate acid solution $c(H_2SO_4)$ = 0.1 mol L^{-1} (State Pharmacopoeia of Ukraine).

Preparing the work solution of potassium hydrogen peroxomonosulphate of $2 \cdot 10^{-2}$ mol L^{-1}. The sample weight 0.615 g of the salt was dissolved in 100.0 ml of the twice distilled water at 293 K. The solution concentration was controlled by the iodometric titration method. For those purposes, 10 ml of the obtained solution were taken using the pipette and transferred to the volumetric flask of 100 ml and the volume was brought up to the mark. 1 ml 0.1 mol L^{-1} of the sulphate acid and 1 ml of 1% KI were added to 10.00 ml of the obtained solution in the conical flask of 75 ml and thoroughly stirred. The isolated iodine was titrated by 0.02 mol L^{-1} of the sodium thiosulphate solution by measuring the volume using the semimicroburette of 10 mL.

RESULTS AND DISCUSSION

The reaction kinetics was studied and stoichimetry of S-oxidation reaction of semisynthetic penicillin such as ampicillin trihydrate and sodium oxacillin individually and in the mixture of "Ampiox" preparation was calculated using potassium hydrogenperoxomonosulphate in water solutions at pH 3-6: 1 mol of every penicillin per 1 mol of $KHSO_5$ (formation of appropriate sulfoxides), quantitative interaction is obtained during 1 min (observation time) by the method of iodometric titration. These allowed developing new procedure of quantitative determination of penicllin summary content by the method of reverse iodometric titration of oxidant excess using potassium hydrogenperoxomonosulphate as analytical reagent.

The transformations scheme that is the base of analytical determination is given below:

$R_2S + KHSO_5 = R_2S{=}O + KHSO_4$

$KHSO_5 + 2KI + H_2SO_4 = I_2 + KHSO_4 + H_2O + K_2SO_4$

$I_2 + 2Na_2S_2O_3 = 2\ NaI + Na_2S_4O_6$.

The procedure of iodometric determination of penicillins' sum in the "Ampiox" preparation. About 0.4 g (exact sample weight) of the powder of the "Ampiox" preparation is to be transferred to the volumetric flask of 250 mL, added 8 ml of dimethylformamide, brought by the bidistillate up to the mark at 20 C°, thoroughly stirred and filtered using the paper filter with the red strip. 20 mL of the obtained solution are to be taken using the pipette and transferred to the volumetric flask of 100 ml, added 10 mL of the potassium hydrogen peroxomonosulphate solution of $2 \cdot 10^{-2}$ mol L^{-1}, are to be shaken thoroughly within 1 minute, and the solution volume is to be brought up to the mark and stirred again. After that, 10 mL of the obtained mixture are to be taken using the pipette and transferred to the conical flask of 75 mL, acidified by 1 ml 0.1 mol L^{-1} of H_2SO_4 solution, 2 mL 5 % KI solution are to be added and the isolated iodine is to be titrated by 0.02 mol L^{-1} of sodium thiosulphate solution.

In parallel and under the same conditions, the control experiment is to be performed with the same quantity of the potassium triple salt of peroxymonosulfuric acid. The content of the penicillins in the solution c_0 in mole/l is being calculated by the formula:

$$c_0 = \frac{V_0 - V_1}{2 \cdot 20} \cdot 0.02 \cdot K \cdot \frac{\bar{a}}{a},$$

where V_0 – the volume of the standard $Na_2S_2O_3$ solution, expended for the titration in the control experiment, ml;
V_1 – the volume of the standard $Na_2S_2O_3$ solution, expended for the titration in the operational experiment, ml;
20 – the aliquot volume of the solution of the preparation, taken for the analysis;
a – the sample weight of the examined powder of the preparation of this series, taken for the analysis, g;
$\bar{a}$ – the average weight of the preparation in the capsule, g;
0.02 – concentration of the $Na_2S_2O_3$ solution, mol L^{-1};
K – correcting factor of the sodium thiosulphate concentration up to 0.0200 mol L^{-1}.

Scheme of transformations that give the reaction product (ampicillin trihydrate as the example) is given on the Fig. 1.

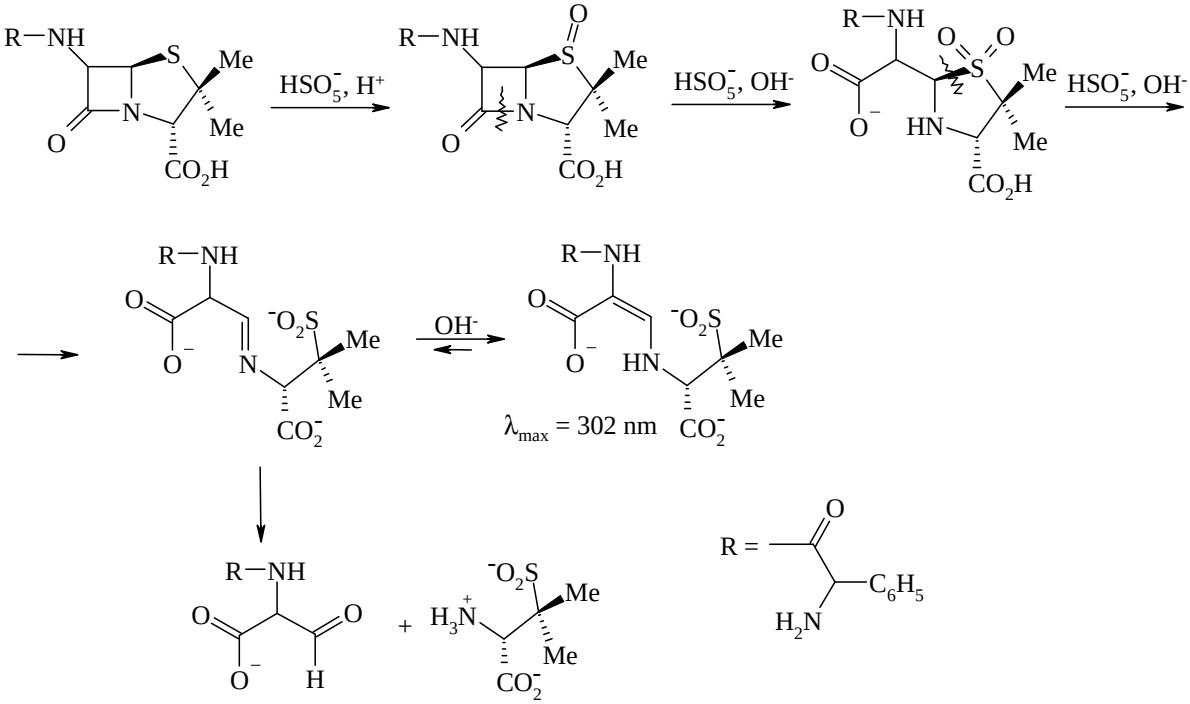

Fig. 1. Scheme of conjugated reactions of peroxiacidic oxidation and perhydrolysis of ampicillin with the formation of substituted derivate of *N*-acryl-*β*-penicilamine sulfinate (IV)

During the experiment it was determined that the mixing order has the significant influence on the kinetics and reaction product. The greatest rate of product accumulation was observed only after preliminary mixing of standard solution of penicillin under study with hydrogenperoxomonosufate and further addition of alkali solution. The

maximal activity of hydrogenperoxomonosulphate in the reaction was obtained under the concentration of $2 \cdot 10^{-2}$ mol L^{-1}. It was determined that the optimal alkali concentration under which the greatest rate of product accumulation was observed was $(5\text{-}7) \cdot 10^{-3}$ mol L^{-1}. Without hydrogenperoxomonosulphate in the given conditions during the first 30 min (observation time) the product formation was not proceeding. This necessary excess of potassium hydrogenperoxomonosulphate can be explained by its participation in the process of further hydrolytic cleavage of the formed on the first reaction stage corresponding S-oxides of ampicillin and oxacillin in basic medium (nucleophilic catalysis of *β*-lactam and tiazolidin cycles hydrolysis). Electronic spectra of light adsorbance of the formed product under the reaction of ampicillin trihydrate sulfoxide product in time are given on Fig. 2.

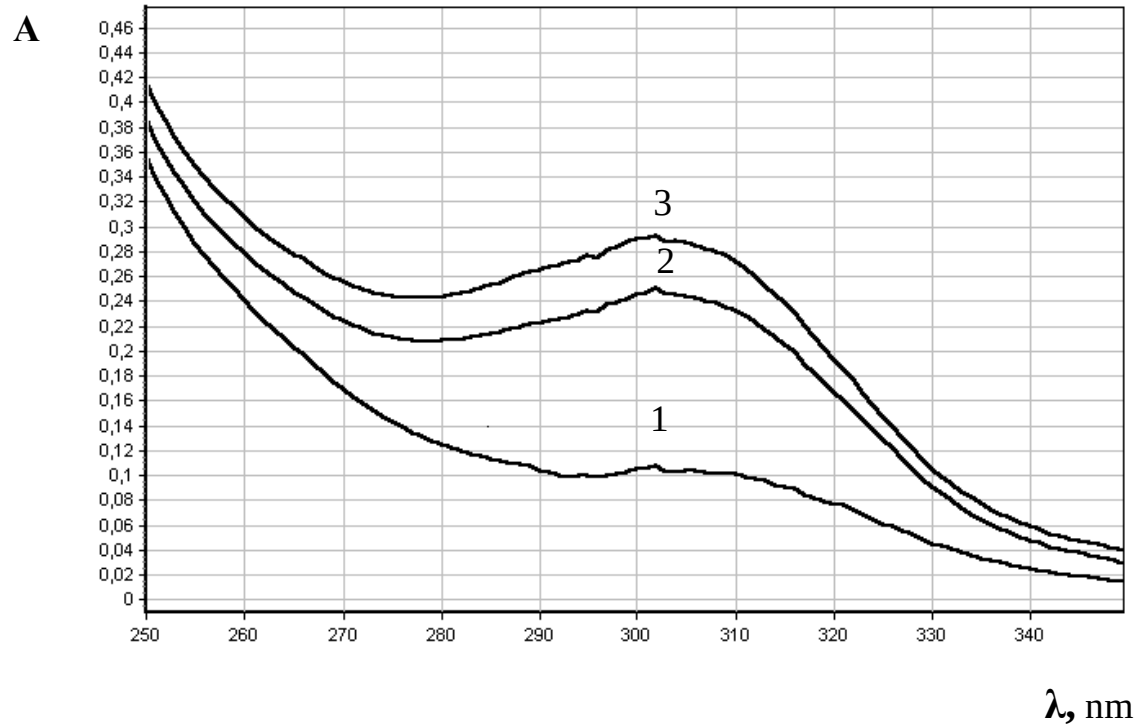

Fig. 2. Electronic spectra was light adsorbance of the formed product under the reaction of ampicillin trihydrate sulfoxide product in time: 1 – 1 min, 2 – 5 min, 3 – 10 min. *c*(NaOH) = $7.3 \cdot 10^{-3}$ mol L^{-1}; *c*($KHSO_5$) = $2 \cdot 10^{-3}$ mol L^{-1}; *c*(Amp.*t*/h) = 25 μg mL^{-1}

On the Fig. 3 the electronic spectra of the product of conjugated reaction of peroxiacidic oxidation, alkali hydrolysis and pehydrolysis of oxacillin that are recorded in time during the reaction (equilibrium period).

The formation of characteristic wave with maximum under certain wavelength cogently shows the formation of one or several similar by the structure reaction products, that possibly belong to substituted derivates of N-acryl-β-penicillamin sulphinate (IV) [11].

Kinetic dependence of light adsorbance of penicillin solutions in time that have the view of curves with saturation with linear parts on the initial reaction stage are given on the Fig.4.

The calibration plot of quantitative determination of ampicillin trihydrate is given on the Fig. 5.

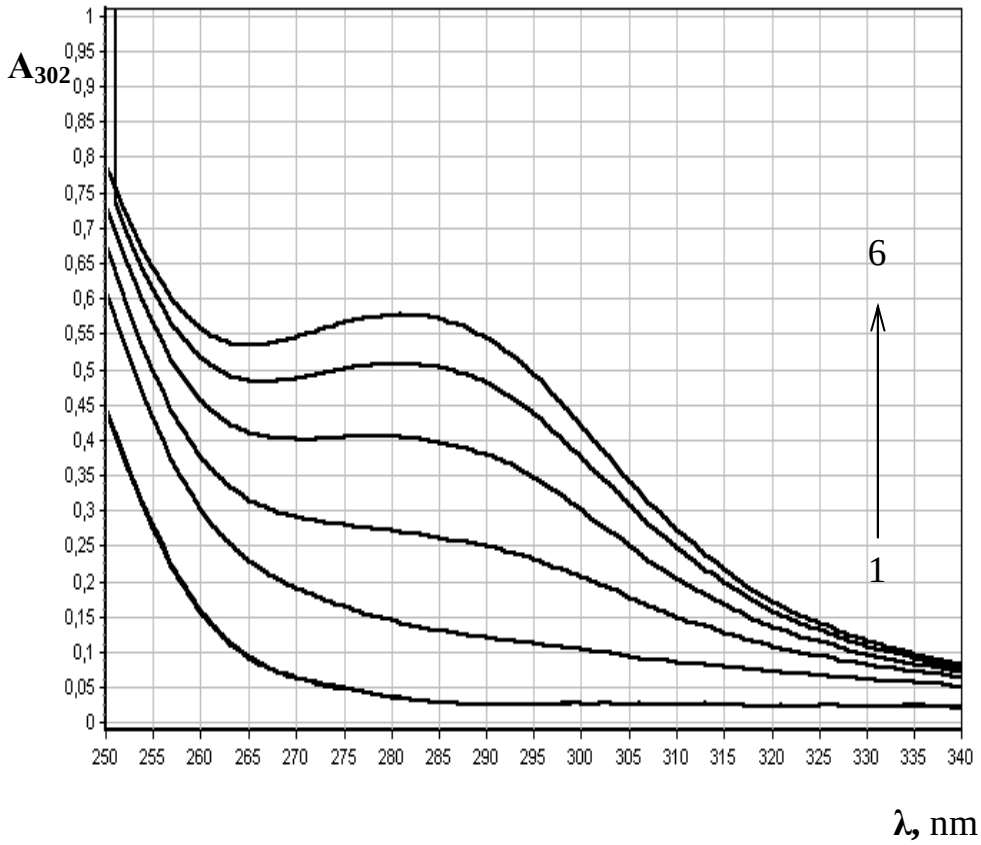

Fig. 3. Electronic spectra of light adsorbance of the product of alkali hydrolysis (1) and perhydrolysis of sodium oxacillin (2-6) in time, min: *1* – 0–20, *2* – 5, *3* – 10, *4* – 15, *5* – 20, *6* – 25; *c*(NaOH) = $6.1 \cdot 10^{-3}$ mol L^{-1}; *c*($KHSO_5$) = $1.2 \cdot 10^{-3}$ mol L^{-1}; *c*(Na-oxac.) = 30 μg mL^{-1}

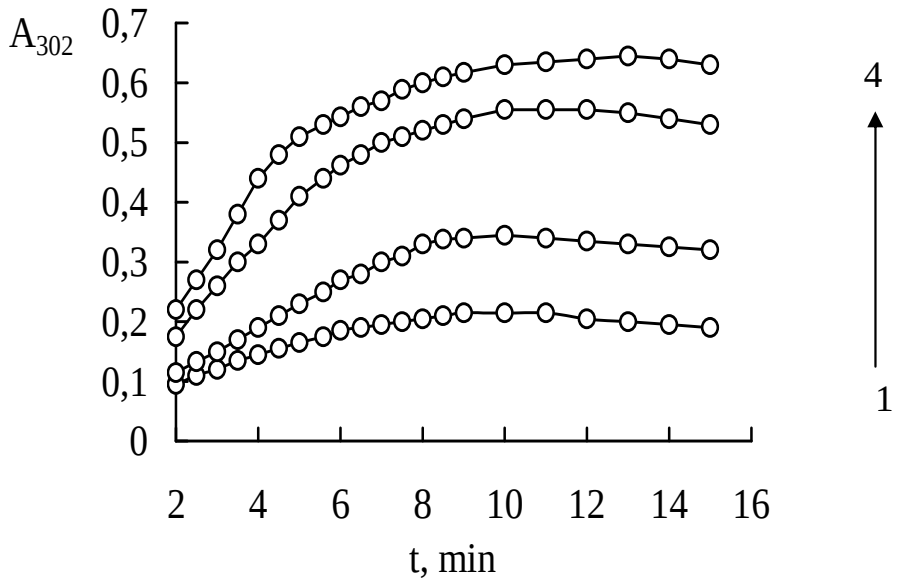

Fig. 4. Effect of ampicillin trihydrate on the perhydrolysis reaction kinetics. *c*($KHSO_5$) =$2 \cdot 10^{-3}$ mol L^{-1}; . *c*(NaOH) =$5 \cdot 10^{-3}$ mol L^{-1}. *c*(Amp. t/h), μg mL^{-1}: *1* - 10; *2* - 20; *3* - 30; *4* – 40

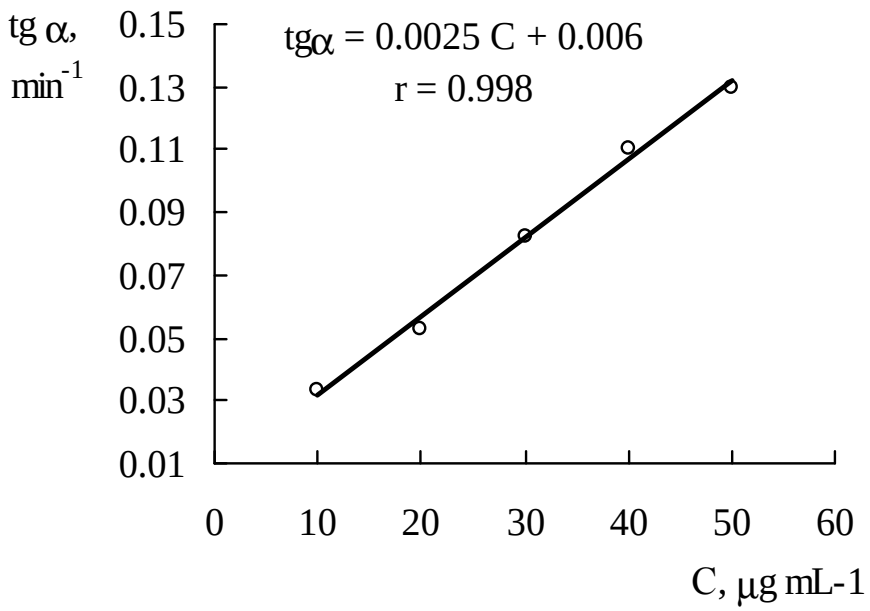

Fig. 5. Calibration plot of quantitative determination of ampicillin trihydrate by spectrophotometric method. $c(KHSO_5) = 2 \cdot 10^{-3}$ mol L^{-1}; . $c(NaOH) = 4.9 \cdot 10^{-3}$ mol L^{-1}

The results of model mixtures analysis with the known content of ampicillin trihydrate and medicinal preparation "Ampiox" are given in Table 1 and 2 respectively.

Table 1 The results of the quantitative determination of ampicillin in the model mixture based on the reaction with the peroxomonosulphate acid (*n*=5, P=0.95)

Penicillin taken, mg	Determined		Metrological characteristics
	mg	%	
mixture 250 mg (ampicillin 125.0 mg + oxacillin 125.0 mg)			
ampicillin 125.0	127.9 124.2 123.1 126.9 128.1	102.3 99.4 98.5 101.5 102.5	$\bar{x}$ = 126.0 (100.8 %) *S*=±2.26 $S_{\bar{x}}$ =±1.01 $\Delta\bar{x}$ =±2.81 RSD = ±1.8% ε =±2.2% δ = + 0.8 %
oxacillin 125.0	121.8 123.3 126.3 122.1 126.8	97.4 98.6 101.0 97.7 101.4	$\bar{x}$ = 124.1 (99.3 %) *S*=±2.35 $S_{\bar{x}}$ =±1.05 $\Delta\bar{x}$ =±2.92 RSD = ±1.9% ε =±2.35% δ = – 0.75 %

The methodology of the quantitative determination of the content of ampicillin trihydrate in the "Ampiox" preparation by the kinetic method. About 0.4 g of the content of the "Ampiox" capsule (exact sample weight) are to be added to the volumetric flask of 250 mL, added 8 mL of dimethylformamide, brought up to the mark at 20 C^o using the distilled water, shaken thoroughly and filtered using the paper filter with the red strip. 5.00 mL of the obtained solution are to be taken using a microburette to the volumetric flask of 50 mL, added 5 mL $2 \cdot 10^{-2}$ mol $L^{-1}$of the peroxomonosulphate acid solution, shaken thoroughly and left for 1 min. 5.0 mL $1.6 \cdot 10^{-}$ mol L^{-1} of sodium hydroxide solution are to be added, the volume is to be brought up to the mark using the distilled water and thoroughly stirred. Upon adding the alkali solution, the countdown is to be started and the seconds counter is to be on. The maximal light absorbance of the reaction product is observing at 302 nm. The obtained solution is to be photometered in the quartz cell with the thickness of 1 cm against the distilled water (compensation solution) every minute within 10 min. at 20 °C and the

kinetic curve of the dependence of the optical density A upon the time is to be graphed. The slope ratio of the linear section of the kinetic curve is to be found upon the graph in the experiment with the work sample.

About 0,15 g of the substance of ampicillin trihydrate (exact sample weight) are to be put to the volumetric flask of 250 mL, 3 mL of dimethylformamide are to be added, brought up to the mark at 20 Cº using the distilled water, thoroughly stirred and filtered using the paper filter with the red strip. After that, the analysis is to be performed in the same way like the first one. The slope ratio of the kinetic curve in the experiment with the work standard of ampicillin trihydrate, min^{-1} is to be found.

The content of $C_{16}H_{19}N_3O_4S$, in g, in one capsule (X_{amp}) is being calculated by the formula:

$$X_{\text{amp}} = \frac{a_{st} \cdot tg\alpha \cdot \bar{a}}{a \cdot tg\alpha_{st}},$$

where a_{st}– the sample weight of the work standard of ampicillin trihydrate, g;

$tg\alpha_{st}$ – the slope ratio of the kinetic curve in the experiment with the work standard of ampicillin trihydrate, $min.^{-1}$;
a – the sample weight of the studied powder of ampicillin trihydrate, g;
$\bar{a}$ – the average weight of the tablet, g;
$tg\alpha$ – the slope ratio of the kinetic curve in the experiment with the studied ampicillin trihydrate solution, $min.^{-1}$.

The content of oxacillin in the medical form is to be found based on the difference between the total content of penicillins and the content of ampicillin, obtained by the kinetic method.

Table 2 The results of the quantitative determination of ampicillin in the "Ampiox" preparation based on the reaction with the peroxomonosulphate acid (*n*=5, P=0.95)

Penicillin taken, mg	Determined		Metrological characteristics
	mg	%	
capsules of 250 mg of series 291110, manufactured by "PHARMACEUTICAL COMPANY "ZDOROVIE" LTD., Kharkiv, Ukraine.			
ampicillin 124.7*	124.5 128.2 124.5 127.2 128.1	100.2 103.1 100.2 102.3 103.1	$\bar{X}$ = 126.5 (101.2 %) S=±1.92 $S_{\bar{X}}$ =±0.86 $\Delta\bar{x}$ =±2.39 RSD = ±1.5% ε =±1.9% δ = + 1.4 %
oxacillin 124.3**	123.9 123.3 125.3 121.1 124.9	102.6 99.7 102.1 99.5 101.8	$\bar{X}$ = 123.7 (98.9 %) S=±1.66 $S_{\bar{X}}$ =±0.74 $\Delta\bar{x}$ =±2.05 RSD = ±1.4% ε =±1.7% δ = – 0.5 %

*Notes: * – the content of ampicillin, specified in the quality certificate;*
*** – the content of oxacillin, specified in the quality certificate.*

CONCLUSION

1. The reaction kinetics of the peroxyacidic oxidation and perhydrolysis of ampicillin and oxacillin with potassium peroxomonosulphate in the alkaline medium is studied.
2. As an oxidizing agent, the potassium triple salt of peroxymonosulfuric acid, $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$, syn. "Oxone", was applied.
3. The procedure was developed and the possibility of the quantitative determination of ampicillin and oxacillin in the "Ampiox" preparation based on the results of the kinetic-spectrophotometric and iodometric methods with potassium peroxomonosulphate as reagent was shown. RSD ≤ 1.5 % (δ =+1.4…− 0.5 %).

REFERENCES

[1] MYe Blazheyevskyi. *Pharm. J.* **2003**, 5, 66-78.
[2] The State Pharmacopoeia of Ukraine, The 3-rd issue, Kh.: RIREG, **2008**, 285-289.

[3] KV Zaitseva; VG. Alexeiev. *The Bulletin of the Tver State University*, **2007**. 2 (Ser. Chem. 3), 112-115.
[4] M Turkevych; O Vladzimirs'ka; R Lesyk. Pharmaceutical Chemistry (steroid hormones, their synthetic substitutes and heterocyclic compounds as medicinal agents), Vinnytsia: "Nova knyga", **2003**, 2003. – 464.
[5] F Bruno; R Curini; A Corcia et al. *J. Agric. Food. Chem*, **2001,** 49 (7), 3463-3470.
[6] A Fernández-González; R Badía; ME Díaz-García. *J. Pharm. Biomed. Anal*. **2002**, 29 (4), 669-679.
[7] E Gremaud; R Mohamed; Y Hammel. *J. Chromatogr*, **2008,** 1177 (1), 58-76.
[8] A Kaufmann; P Butcher; K Maden et al. *J. Chromatogr. A.*, **2004**, 1042 (1), 107-111.
[9] E Mendez-Alvarez; R Soto-Otero; G Sierra-Paredes . et al. *Biomed. Chromat*., **2009**, 5 (2), 78-82.
[10] MY Blazheyevski; YY Labuzova. *J. Chem. Pharm. Res.*, **2013**, 5(8), 115-121.
[11] D Rogič. *Acta Pharm. Jugosl*., **1985**, 35, 47–52.
[12] Medikondu Kishore; Y Hanumantharao. *J. Chem. Pharm. Res.*, **2010**, 2(4), 99-105.