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Research Article

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Quantitative determination of amoxicillin trihydrate in medical forms using kinetic method

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ABSTRACT

The kinetic of conjugated reactions of S-oxidation and perhydrolysis of amoxicillin with potassium hydrogenperoxomonosulfate in alkaline medium is studied by the increase of forming product light absorbance at 296 nm. The procedure of the quantitative analysis of amoxicillin in capsules, pills and suspension by spectrophotometric-kinetic method is elaborated using triple potassium Caro's salt solution («Oxone») as a reagent. RSD ≤ 2.00 %. The obtained results have good agreement with the Pharmacopoeia one δ = (+0.1…–1.3) %.

Key words: kinetic method of analysis, amoxicillin, potassium hydrogenperoxomonosulfate

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INTRODUCTION

Amoxicillin is a penicillin's antibiotic. It plays an important role in the therapy of infections [10]. Preparations of penicillin family are derivates of 6-aminopenicillanic acid (6-APA), a condensed system of thiazolidine and *β*-lactam tetramine cycles that differ by the acyl radical R connected with 6-APA aminogroup.

Different methods, such as biological, chemical and physicochemical are recommended for its quantitative determination.

Biological methods are based on the direct antibiotic biological action on a test-microorganism sensitive to the given antibiotic. Disadvantages of the biological methods are the long-lasting procedure and the dependence of the results precision on the external factors.

The extensive literature survey reveals varies methods of quantitative determination of penicillin family preparations, such as HPLC [12, 20, 21], spectrophotometry[8, 10, 17], iodometry [10], potentiometric titration [6]; for amoxicillin determination potentiometric method with ISE [9], different variants of voltammetry [22], amperometry [13], polarography [4] and kinetic analysis [5, 19] are proposed.

The spectrophotometric methods that are based on the application of phenol Folin–Ciocalteu reagent [2,3], reactions with Copper (II) salts [1] and etc. [11, 14, 15, 16] are also known. These methods give the possibility to determine penicillin in medical preparations in presence of different excipients.

Thus, the improvement of the known and development of new methods of quantitative determination of penicillin is rather important. The existing pharmacopoeial methods of penicillin preparations determination are quite complex, long-lasting and require the application of complex and expensive devices [10]. The disadvantage of the known simple enough in performance methods of spectrophotometric determination of penicillin, which are based on the determination of the final products of their hydrolytic cleavage, is the requirement of prolonged heating.

The developed method of amoxicillin kinetic determination has several advantages: makes it possible to identify the preparation in much smaller quantities than the pharmacopoeial iodometric method, it is applicable to the same range of concentrations, as in photometric determination of hydrolysis products, but it doesn't require prolonged heating of the reaction mixture, it is simpler and faster than the method of chromatographic analysis.

It is based on the preliminary oxidation of amoxicillin with potassium hydrogenperoxomonosulfate excess to the corresponding S-oxide, followed by determination of the hydrolytic conversion of its product in an alkaline medium by the kinetic spectrophotometric method. The scheme of transformations that leads to the formation of the reaction product, probably derived N-substituted acrylic-β- penicillamine sulfinate (IV), is shown below in Figure 1.

EXPERIMENTAL SECTION

Reagents and Chemicals

All the materials were of analytical reagent grade, and the solutions were prepared with double-distilled water.

Amoxicillin trihydrate (2S,5R,6R)-6-{[(2R)-2-amino-2-(4-hydroxyphenyl)-acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid in pills "Ospamox", 500 mg of drug substance, serial No. 158399, produced by «Sandoz GmbH», Austria, in capsules "Gramox-A", 500 mg of drug substance, serial No. 081109, produced by "Sperko Ukraine", Kyiv, Ukraine, about 20 g of granulate for preparation of 60 mL of suspension for internal use of the following content: 250 mg of amoxicillin trihydrate in 5 mL of suspension, serial No. 050609, produced by "Sperko Ukraine", Kyiv, Ukraine.

Peroxomonosulfate acid was used as an oxidant in the view of a triple potassium salt ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) of an "extra pure" grade. The commercial name is «Oxone» with the content of active Oxygen ≤ 4.5 %. It is available, has good solubility and stability in water. It was proposed for cefodraxil kinetic spectrophotometric determination as an analytical reagent [23].

Preparation of Standard Solution

Standard solution of amoxicillin trihydrate (500 μg mL^{-1}): 500 mg of amoxicillin trihydrate was diluted in 2.0 mL of dimethylformamide (DMF), transferred in 100 mL volumetric flask and diluted to the mark with double distilled water at +20 °C.

Standard solution of Peroxomonosulfate ($2 \cdot 10^{-2}$ mol L^{-1}): 0.615 mg of Oxon was transferred in 100 mL volumetric flask and diluted to the mark with double distilled water at +20 °C. The solution of peroxomonosulfate was standardized iodometrically.

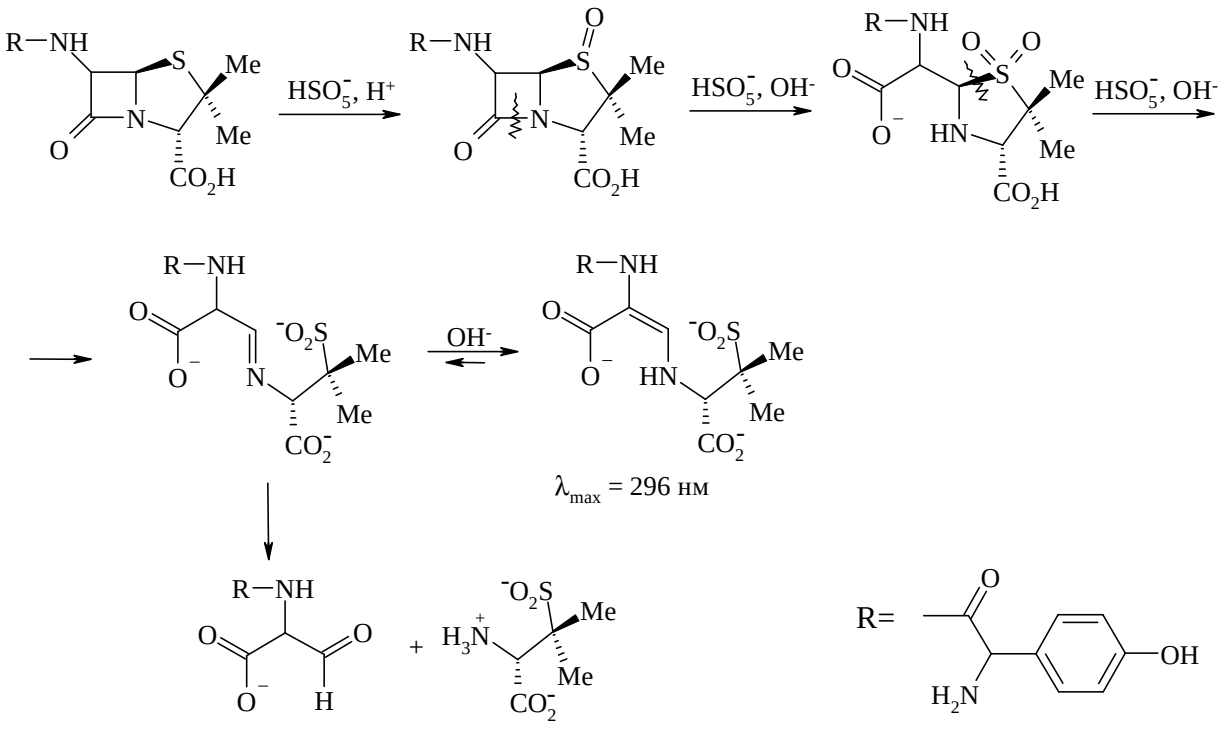
Equipment

Electrochemical measurements were carried out in the spectrophotometer SP-26 (LOMO); kinetics was studied by the produced product light absorbance at 296 nm. The optical density of the solution was studied in the cell with a thickness of absorbing layer l = 1 cm. Solutions were thermostated in UTU- 2 («Zeamit», «Horizont Krakow-Poland») before mixing, time was recorded using stopwatch after mixing. The 0.1 mol L^{-1} solution of sodium hydroxide without carbonates was used to create and maintain the required acidity. Processing of the results was carried out by "tangents" (the differential version) method. Rate was estimated by the slope of the linear section of the kinetic curve A – time (tg α_{amp}, in min^{-1}).

RESULTS AND DISCUSSION

The results of the experiment showed that the order of mixing influences on the kinetics and yield of the reaction. The highest rate of product accumulation was observed only after prior mixing of the sample of amoxicillin under study with potassium hydrogenperoxomonosulfate, and therefore with alkali solution. Maximum activity of potassium hydrogenperoxomonosulfate in the reaction was achieved at concentrations $2 \cdot 10^{-3}$ mol L^{-1}.

The scheme of conjugated reactions of peroxiacidic oxidation and perhydrolysis of amoxicillin trihydrate with production of substitute derivate of N-acryl-β-penicilamine sulfinate (IV)



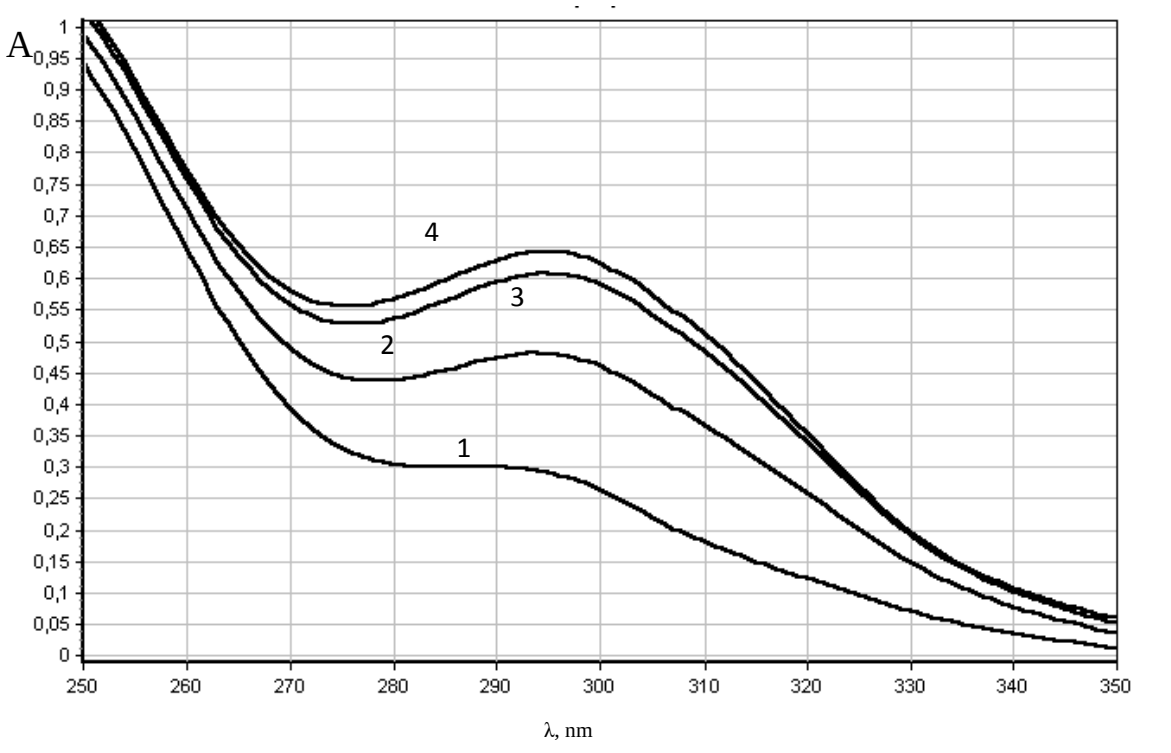


Fig. 1. Light absorbance electronic spectra of alkali hydrolysis (1) and perhydrolysis (2–4) reactions of amoxicillin trihydrate product in time: 1 – 0–20 min, 2 – 5 min, 3 – 10 min, 4 – 15 min

$c(NaOH) = 6.1 \cdot 10^{-3}$ mol L^{-1}; $c(KHSO_5) = 2{,}0 \cdot 10^{-3}$ mol L^{-1}; c(amox. t/h) = 30 μg mL^{-1}

It was determined experimentally that the optimum concentration of alkali in which the rate of the reaction product formation is the highest is $6 \cdot 10^{-3}$ mol L^{-1}. Without potassium hydrogenperoxomonosulfate in the same conditions during the first 30 min (observation time) reaction product is not formed. This required excess of potassium hydrogenperoxomonosulfate can be explained by its participation in the process of further hydrolytic cleavage - perhydrolysis reaction of the formed on the first stage corresponding amoxicillin S-oxide in strong alkaline medium (nucleophilic catalysis of the *β*-lactam and thiazolidyne cycles hydrolysis) (see scheme).

Light absorbance electronic spectra of alkali hydrolysis (1) and perhydrolysis (2–4) reactions of amoxicillin trihydrate product in time are given on Fig. 1 As can be seen, the maximum absorption of the formed product is observed at 296 nm. Therefore, this wavelength was chosen as analytical for the reaction kinetics study. The reaction rate was estimated by slope of initial section of the kinetic curves constructed in coordinates light absorbance A against time.

The calibration graph of amoxicillin assay is given on Fig. 2. It shows that in the range of 5 to 50 μg mL^{-1} concentration dependence of tgα, which is proportional to the reaction rate, is linear. This fact makes it possible to carry out the method of amoxicillin determination processed in the specified concentration range using the standard method.

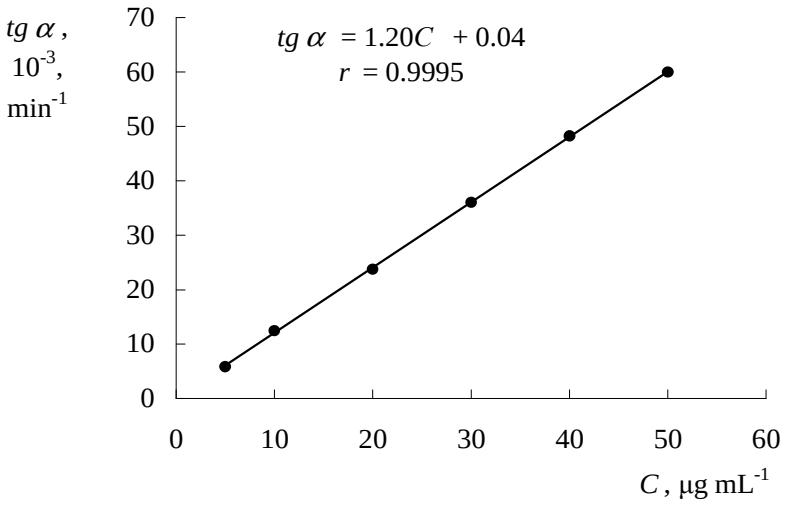


Fig. 2. Calibration graph of amoxicillin t/h assay
c(KHSO$_5$) = 2·10^{-3} mol L^{-1}; c(NaOH) =1.6·10^{-3} mol L^{-1}

Construction of the calibration graph. Aliquots of 0.5–5.50 mol L^{-1} of amoxicillin standard solution were pipetted into a series of 50 mL volumetric flask containing 5.0 mL of 0.02 mol L^{-1} $KHSO_5$ solution, mixed thoroughly and left for a minute. Then in each flask 5.0 mL of 0.06 mol L^{-1} sodium hydroxideis solution was added, diluted to the mark with distilled water and mix thoroughly. After the addition of alkali solution the stopwatch was turned on. The resulting solutions were measured photometrically in a quartz cuvette at 296 nm against distilled water (compensation solution) during first 10 min every minute at 20° C and the absorbance kinetic curves against time were constructed. According to the inclination of the linear graphs of kinetic curves the calibration dependence tgα against amoxicillin concentration (C, μg mL^{-1}) were plotted.

Procedure of amoxicillin assay in capsules. 500 mg (precise weight) of the studied drug was transferred in 100 mL volumetric flask containing 2.0 mL of DMFA solution and diluted to the mark with distilled water. The content of mixture was mixed well. 3.0 mL of obtained solution was transferred in 50 mL volumetric flask, further as while calibration graph construction. The resulting solution was measured photometrically in a quartz cuvette at 296 nm against distilled water (compensation solution) during first 15 min every minute and the absorbance kinetic curves against time was constructed. The slope of the linear section of the kinetic curve, tgα was determined.

Procedure of amoxicillin assay in pills. 671 mg (powdered pills) of the studied drug was transferred in 100 mL volumetric flask containing 2.0 mL of DMFA solution and diluted to the mark with distilled water. The content of mixture was mixed well and filtrated. 10.0 mL of obtained solution was transferred in 100 mL volumetric flask, further as for capsules procedure.

Procedure of amoxicillin assay in suspension. 671 mg (powdered pills) of studied drug was transferred in 100 mL volumetric flask containing 2.0 mL of DMFA solution and diluted to the mark with distilled water. The content of mixture was mixed well and filtrated. 10.0 mL of obtained solution was transferred in 50 mL volumetric flask, further as for capsules procedure.

Content of $C_{16}H_{19}N_3O_5S$, in mg, ($x_{amox.}$) is calculated by the equation:

$$x_{amox} = \frac{a_{st} \cdot \mathrm{tg}\alpha \cdot w \cdot 0{,}8713 \cdot \overline{a}}{a \cdot \mathrm{tg}\alpha_{st}},$$

where:

a_{st} – weight mass of amoxicillin trihydrate standard solution, mg;

$\mathrm{tg}\alpha_{cm}$ – the slope of the linear section of the kinetic curve of amoxicillin trihydrate standard solution, min^{-1};

w – mass fraction of amoxicillin trihydrate standard solution main substance;

a – weight mass of amoxicillin trihydrate solution under study, mg;

$\overline{a}$ – average mass of the drug in a capsule, pill, vial, mg;

$\mathrm{tg}\alpha$ – the slope of the linear section of the kinetic curve of amoxicillin trihydrate solution under study, min^{-1};

0.8713 – conversation coefficient for amoxicillin anhydrous.

The results of amoxicillin kinetic determination using the reaction with potassium hydrogenperoxomonosulfate are given in Table 1.

Table 1 The results of amoxicillin kinetic determination using the reaction with potassium hydrogenperoxomonosulfate (P=0.95, n=5)

Taken, mg	Found		Metrological characteristics
	mg	%	
Ospamox – pills, 500 mg, «Sandoz GmbH», Austria			
492.0*	486.7 488.2 492.05 497.0 499.5	97.3 97.6 98.4 99.4 99.9	$\overline{X}$ = 493 (99%) S=±5.5 S_x = ±2.5 $\Delta\overline{x}$ =±6.8 S_r = ±1.1% ε = ±1.4% δ = + 0.1 %
Gramox-A – granulate for suspension preparation "Sperko Ukraine", Kyiv, Ukraine (250 mg of amoxicillin trihydrate in 5 mL of suspension)			
250.8*	243.6 248.6 250.6 249.5 244.6	97.4 99.4 100.2 99.8 97.8	$\overline{X}$ = 247 (99%) S=±3.1 S_x = ±1.4 $\Delta\overline{x}$ =±3.8 S_r = ±1.25% ε = ±1.55% δ = –1.3 %
"Gramox-A"– 500 mg of drug substance "Sperko Ukraine", Kyiv, Ukraine			
496.0*	491.8 479.8 501.6 499.8 512.5 497.2 496.6	99.15 96.7 101.1 100.8 103.3 100.2 100.1	$\overline{X}$ = 497 (99%) S = ±9.9 S_x = ±3.8 $\Delta\overline{x}$ = ±9.2 S_r = ±2.0% ε = 1.85% δ = +0.1 %

**Amoxicillin content in the certificate (BPh 2009)*

As it is seen from the results the amoxicillin quantitative determination is possible in different medical forms with appropriate precision: RSD ≤ 2.0 %, δ = (+0.1…–1.3) %.

CONCLUSION

1.The kinetic of conjugated S-oxidation and perhydrolysis reactions of amoxicillin using potassium hydrogenperoxomonosulfate in aqueous solution was studied.

2. The conditions were optimized, the procedure was developed and the possibility of amoxicillin assay using potassium hydrogenperoxomonosulfate by the kinetic spectrophotometric method was shown. RSD ≤ 2.00 %. The obtained results have good agreement with the Pharmacopoeial one δ = (+0.1…–1.3) %.

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