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Research Article

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Quantitative determination of phenoxymethylpenicillin using kinetic method

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ABSTRACT

The kinetic of conjugated reactions of S-oxidation and perhydrolysis of phenoxymethylpenicillin with potassium hydrogenperoxomonosulfate in alkaline medium has been studied by the light absorbance increase of a forming product at 269 nm. The procedure of the quantitative analysis of phenoxymethylpenicillin in powder and drug by the kinetic spectrophotometric method is elaborated using triple potassium Caro salt solution («Oxon») as a reagent. RSD ≤ 1.9 %.

Key words: spectrophotometry, phenoxymethylpenicillin, potasium hydrogenperoxomonosulfate

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INTRODUCTION

Phenoxymethylpenicillin (PhMP) is an acid-resistant form of the penicillin, which is produced by the *Penicillium notatum* or other related microorganisms. It has antimicrobial action due to the inhibiting of the bacteria separation process, which leads to their death. PhMP is stable in an acidic medium, and thus is not destroyed in the stomach, well absorbed by the internal use dissolving in the alkaline medium of the intestine, creating a long-term and high concentration of penicillin in the blood. These antibiotic drugs have a low toxicity and do not have cumulative properties. It is prescribed by the same parameters as benzylpenicillin: pneumonia, bronchitis, sore throat, infections of soft tissue, scarlet fever, erysipelas, gonorrhea, and other diseases caused by pathogens susceptible to the drug.

It is produced in the form of tablets by 0.1 and 0.25 g; dragee by 0.1 g; powder for the suspension preparation in vials that contains 1.2; 0.6 and 0.3 g of phenoxymethylpenicillin combined with citric acid, sodium benzoate, raspberry essence and sugar. The powder is white with sweet taste and raspberry smell.

The quantitative determination can be performed by the several methods, such as biological, chemical and physicochemical [6]. The disadvantages of the biological methods are the long-lasting procedure and the dependence of the analysis results accuracy on many external factors.

Chemical and physicochemical methods are widely spread nowadays for the kinetic spectrophotometric determination. There are chromatography [4,7,12,16] and spectrophotometric methods [5,10,15]. Extensive literature survey reveals several methods for the penicillin quantitative determination such as potentiometric titration [3], iodometry [6]; amperometry [9], polarography analysis[1], kinetics [2,18] and etc. [8,11,13,14,17,19,20].

Thus, the task of improving existing and development of new methods of quantitative determination of penicillin is still relevant. The well-known pharmacopoeial methods for the penicillin drugs determination are quite complex, time-consuming to prepare and require the application of sophisticated expensive equipment. Most of the known methods of penicillins spectrophotometric determination, which are reduced to the determination of the final products of their hydrolytic cleavage are also long-lasting and require heating.

The proposed method of phenoxymethylpemisillin quantitative determination has several advantages if compare with the already known: it allows to define the drug in much smaller quantities than recommended by the iodometry method; it is suitable for the same range of concentrations definable as in the spectrophotometric method of hydrolysis products, but do not require heating of the reaction mixture, it is easier and faster than the chromatographic analysis method. The developed method of the analysis is based on the preliminary phenoxymethylpenicillin oxidation by potassium hydrogenperoxomonosulfate excess to the corresponding S-oxide, followed by its definition as a product of hydrolytic cleavage in alkaline medium by the kinetic method of the tangents at 269 nm.

EXPERIMENTAL SECTION

Reagents and Chemicals

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

Phenoxymethylpenicillin was used in the form of tablets by 0.25 g of potassium phenoxymethylpenicillin (potassium salt of (2S,5R,6R)-3,3-Dimethyl-7-oxo-6-[(phenoxyacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate) series 30403, produced by VAT "KYIVMEDPREPARAT", corporation «Arterium» Kyiv, Ukraine, and also in the form of tablets by 0.25 g of phenoxymethylpenicillin [(2S,5R,6R)-3,3-Dimethyl-7-oxo-6-[(phenoxyacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, series 51209, produced by OAO "Synthesis", Russia.

Hydrogenperoxomonosulphate acid as triple potassium salt 2KHSO5·KHSO4·K2SO4 (Oxone®) of "extra pure" qualification was used as oxidant. Active oxygen content is 4.5 % (Acros Organics). The reagent was used due to its availability, good solubility and stability in water, also its relatively high oxidation ability

The theoretical scheme of transformation of the reaction product is given on Fig.1.

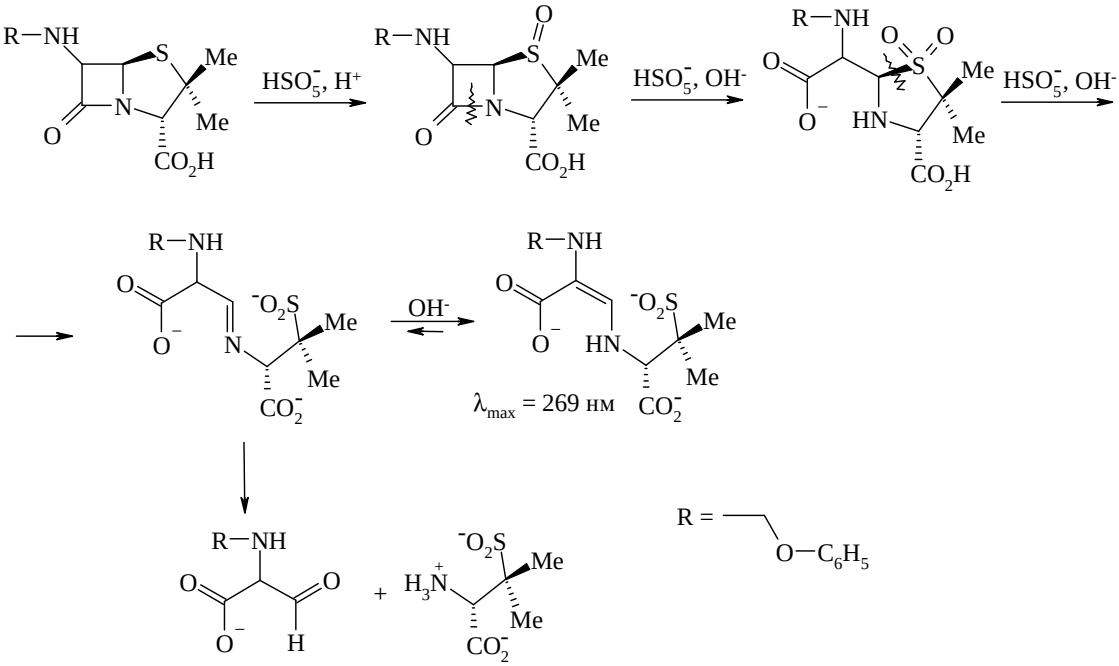

Fig. 1. The scheme of conjugated reaction of peroxiacidic oxidation and perhydrolysis of phenoxymethylpenicillin

Preparing the working standard (WS) solution of potassium phenoxymethylpenicillin *of $1.43 \cdot 10^{-3}$ mol L^{-1}*. The sample weight 0.3958 g of the salt was transferred to the 500 mL volumetric flask, 25 mL of buffer solution with 0.015 mol L^{-1} concentration was added and brought to the mark at 20°C.

Preparing the working standard (WS) solution of potassium hydrogenperoxomonosulfate 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 double-distilled water at 20 ^{o}C. The solution concentration was controlled by the iodometric titration method.

A spectrophotometer SF-46 (LOMO) with 1 cm match quartz cells were used for the spectral measurement. The kinetics of the formed product was studied at 269 nm. The solutions were thermostated in the thermostat UTU-2

(Zeamit, Horizont Krakow-Poland), the time was fixed by the stopwatch after mixing. To create and maintain the required acidity the solution of 0.1 mol L^{-1} sodium hydroxide which did not contain carbonates was used. The initial rate of the reaction at different concentrations was obtained from the slope of the tangent to absorbance time curves.

RESULTS AND DISCUSSION

During the experiment it was determined that the order of mixing influences the kinetics and the yield of the reaction. The highest rate of the product accumulation was observed only after prior addition of potassium hydrogenperoxomonosulfate to phenoxymethylpenicillin, and then to an alkaline solution.

The electronic spectra of interaction product of phenoxymethylpenicillin with reagents depending on the time are shown on Fig. 2.

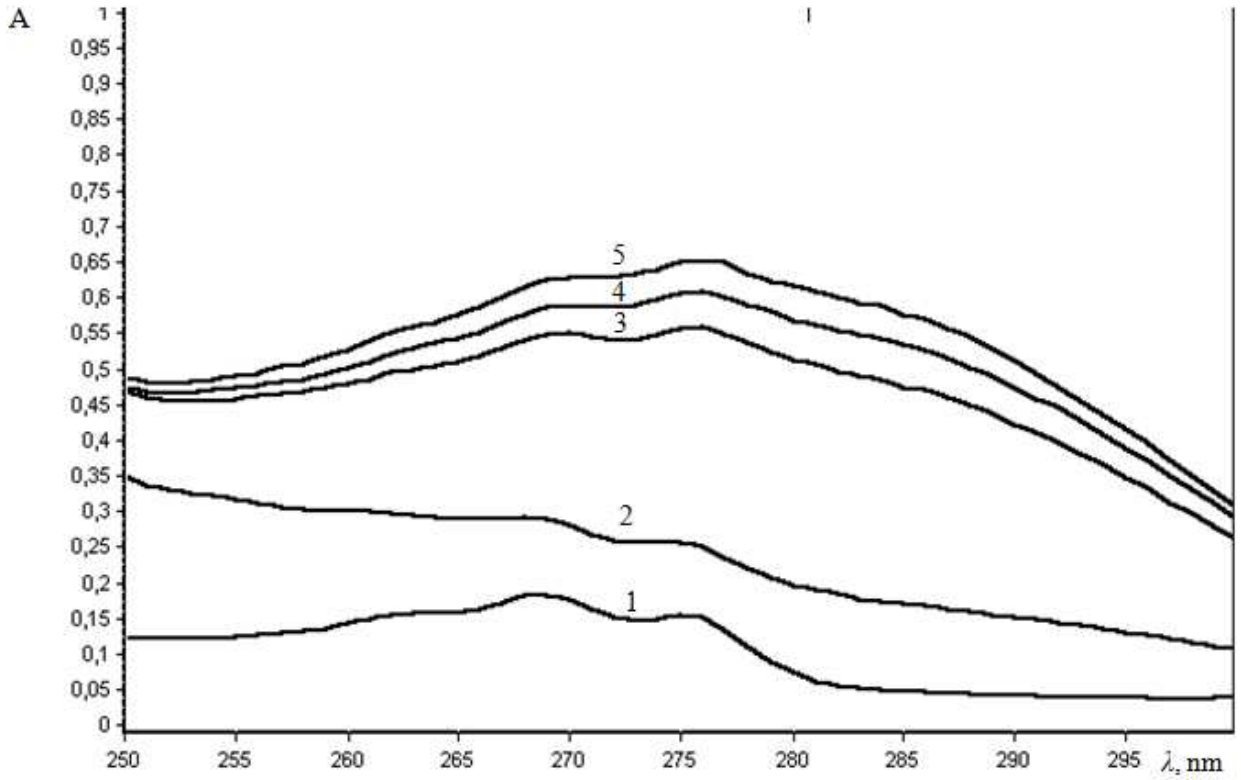

Fig. 2. The electronic spectra of the alkali hydrolysis reaction product (1) and perhydrolysis of phenoxymethylpenicillin absorption (2-5) depending on time, min: 1 – 0–15, 2 – 5, 3 – 10, 4 – 15, 5 – 20. c(NaOH) = $6.1 \cdot 10^{-3}$ mol L^{-1}; c($KHSO_5$) = $2.0 \cdot 10^{-3}$ mol L^{-1}; c(PhMP) = 30 µg mL^{-1}

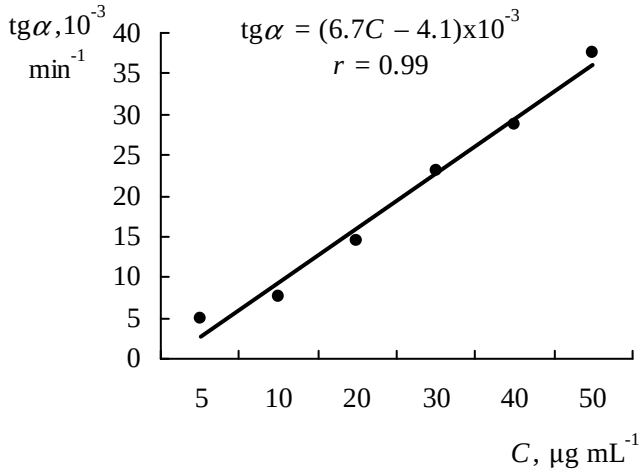

Fig. 3. The calibration plot for kinetic determination of phenoxymethylpenicillin using potassium hydrogenperoxomonosulfate
c ($KHSO_5$) =$2.0 \cdot 10^{-3}$ mol L^{-1}; c (NaOH) =$6.1 \cdot 10^{-3}$ mol L^{-1}

The calibration plot for kinetic determination of phenoxymethylpenicillin in optimum conditions is given on the Fig. 3. The tgα linear concentration dependence was observed within the phenoxymethylpenicillin content in solution 1-45 μg mL^{-1}. This was a precondition for the possibility of using the kinetic method in the drug analysis. The results of the PhMP quantitative determination are given in the Table 1. The proposed method has good accuracy, RSD ≤ 1.9 %.

Initial Rate Method

Aliquots of 0.5–10.00 mol L^{-1} of the studied PhMP test solutions were pipetted into a series of 50 mL volumetric flask containing 5 mL of $2 \cdot 10^{-2}$ mol L^{-1} $KHSO_5$ solution and mixed well. 5 mL of 0.06 mol L^{-1} NaOH solution was added to the flask brought to the mark and missed well. The stopwatch was switched on after the alkali solution addition. The increase in absorbance of the obtained solution at 269 nm was recorded as a function of time for 10 minutes against reagent blank. It shows the dependence of PhMP alkaline solutions absorption against time at 294 nm. They have linear dependence during first 10-15 minutes. The initial rate of the reaction at different concentrations was obtained from the slope of the tangent to absorbance time curves. The calibration graph was constructed by plotting the tangent of the initial rate of the reaction versus concentration of phenoxymethylpenicillin (C, μg mL^{-1}).

Table 1 Results of phenoxymethylpenicillin quantitative determination by the reaction with potassium hydrogenperoxomonosulfate (n=5, P=0.95)

Taken phenoxymethylpenicillin, g	Found		Metrological characteristics
	g	%	
"PHENOXYMETHYLPENICILLIN -KMP", 0.250 g of active substance, series 30403, produced by VAT "KYIVMEDPREPARAT", Kyiv, Ukraine			
0.2389*	0.2405 0.2395 0.2455 0.2463 0.2389	96.2 95.8 98.2 98.5 95.6	$\bar{x}$ = 0.2421 (96.8 %) S = ±0.00349 S_x = ±0.00156 $\Delta\bar{x}$ = ±0.00434 S_r = ±1.44 % ε = ±1.79 % δ = – 1.34 %
"PHENOXYMETHYLPENICILLIN", 0.250g of active substance, series 51209, produced by OAO "Synthesis", Russia			
0.2447*	0.2396 0.2509 0.2495 0.2498 0.2507	95.8 100.4 99.8 99.9 100.3	$\bar{x}$ = 0.2481(99.2 %) S = ±0.00479 S_x = ±0.00214 $\Delta\bar{x}$ = ±0.00596 S_r = ±1.93 % ε = ±2.40 % δ = +1.39 %

** Content of PhMP in preparations was controlled by the independent method of iodometric titration [6].*

Procedure of the phenoxymethylpenicillin quantitative determination. 0.4 g (precise weight) of PhMP was placed in a 500 mL volumetric flask, 25 mL of buffer solution with the concentration of 0.015 mol L^{-1} was added and diluted to the mark with double distilled water. Then, 3 mL of this solution was transferred to 50 mL volumetric flask and further analysis was performed as while calibration graph construction. The obtained solution was transferred to a 1 cm cell to measure the absorbance at the wavelength 269 nm during first 10 min every 2 min against water. A kinetic dependence curve of absorbance A against time, min was obtained. The calculation was performed using the initial rate method (differential variant).

The content of $C_{16}H_{18}N_2O_5S$, in g, in tablets (X_{PhMP}) was calculated using the euqation:

$$X_{PhMP} = \frac{a_{st} \cdot tg\alpha \cdot 0{,}8996 \cdot \bar{a}}{a \cdot tg\alpha_{st}},$$

where a_{st}– phenoxymethylpenicillin WS sample weight, g;
tg α_{st} – kinetic curve slope tangent in the experiment with phenoxymethylpenicillin WS, min^{-1};
a – phenoxymethylpenicillin powder under study sample weight, g;
$\bar{a}$ – tablet average weight, g;
tgα – kinetic curve slope tangent in the experiment with phenoxymethylpenicillin under study, min^{-1};
0.8996 – convertation coefficient from potassium phenoxymethylpenicillin salt to acid.

CONCLUSION

1. The reaction kinetics of the peroxyacidic oxidation and perhydrolysis of phenoxymethylpenicillin with potassium hydrogenperoxomonosulfate in the alkaline medium has been studied.
2. As an oxidizing agent, the potassium triple salt of peroxymonosulfuric acid, $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$, syn. “Oxone”, has been applied.
3.The procedure has been developed and the possibility of the quantitative determination of phenoxymethylpenicillin in 0.25 g tablets based on the results of the kinetic-spectrophotometric method with potassium hedrogenperoxomonosulfate as reagent has been shown. RSD ≤ 1.9 %

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