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Quantitative determination of some penicillin by iodometric method using potassium peroxomonosulphate

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ABSTRACT

Kinetics and S-oxidation reaction of semisynthetic penicillins (amoxicillin trihydrate, ampicillin trihydrate, sodium oxacillin and disodium ticarcillin) by means of potassium hydrogenperoxomonosulphate in aqueous solutions at pH 3-6 was studied using iodometric titration. 1 mol of penicillin is per 1 mol of $KHSO_5$, quantitative interaction proceeds in 1 minute (observation time). The unified procedure was developed and ability of quantitative determination of penicillin by iodometric method using potassium hydrogenperoxomonosulphate ($KHSO_5$) as analytical reagent was shown. RSD ≤ 2. 35 % (δ = -0.1…+1.0%).

Keywords: penicillin, potassium hydrogenperoxomonosulphate

INTRODUCTION

By the chemical structure penicillins are medicinal substances that belong to derivates of 6-aminopenicillanic acid (6-APA). It is a condensed system of tiazolidine and four section azetedine (*β*-lactam) heterocycles, that differs in radical R connected with 6-APA amino group. Their characteristic feature is a rapid bactericide effect on the stage of microorganisms growth and insignificant side effects on human organism. Decomposition of one of the heterocycles leads to complete loss of activity meaning allergic action [1, 2].

Classical iodometry of hydrolysis products is determined to be a basic method of penicillin summary quantitative determination [2, 3]. It's disadvantage is duration at least 40 min, and the necessity in standard samples and in rigid conditions standardization, as iodine interaction with hydrolysis products of penicillin reaction doesn't proceed strictly stoichiometrically: iodine expense, and also the quantity of substance that is equivalent to 1.00 ml 0.005 mol/l (*f*=1/2, I_2) of iodine, depend on the reaction medium temperature [3, 4].

International Pharmacopoeia recommends to determine penicillin summary in semisynthetic penicillin by neutralization method after preparation hydrolysis by excess of sodium hydroxide titrated solution at heating [5].

According to State Pharmacopoeia of Ukraine (SPhU) and European Pharmacopoeia (EPh) penicillin quantitative determination is performed by high performance liquid chromatography (HPLC) [6-8].

The following quantitative procedures of penicillin determination are described: using potentiometry titration and ionometry [9-11], spectrophotometry [12-13], extraction photometry [14], voltammetry [15] and polyarography [16], flow-injection analysis with spectrophotometry [17] and chemiluminescense [18] detection, micelle electrokinetic capillary [19] and paper [20] chromatography, chemiluminescense [21] and kinetic analysis methods [22].

New unified procedures of penicillin (amoxicillin trihydrate, ampicillin trihydrate, sodium oxacillin and disodium ticarcillin) quantitative determination by the reverse titration using potassium hydrogenperoxomonosulphate ($KHSO_5$) as analytical reagent were developed

EXPERIMENTAL SECTION

Peroxomonosulphate acid as triple potassium salt $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$ (Oxone®) of "extra pure" qualification was used as oxidant. Active oxygen content is 4.3 % (Acros Organics). The reagent is used due to its availability, good solubility and stability in water, also its relatively high oxidation ability. Standard electrode potential for semireaction

$$HSO_5^- + 2\ H^+ + 2\ e^- \rightarrow HSO_4^- + H_2O$$

is 1.8 V [23].

Reagents and chemicals

0.02 mol/l potassium hydrogenperoxomosulphate solution and 0.02 mol/l sodium thiosulphate (prepared from fixanal) were used as titrants.

Solution of potassium iodide (5 %) was prepared by dissolving 1.0 g of potassium iodide in just boiled distilled water transferring the solution into a 100-ml volumetric flask, diluting to volume and mixing.

Solution of sulphur acid, $c(H_2SO_4)$ = 0.1 mol/l.

Titrant volume is determined by 10 ml micro burette with precise ±0.01 ml.

Solution of potassium hydrogenperoxomonosulphate (0.02 mol/l) in water was prepared by dissolving 0.615 g of potassium hydrogenperoxomonosulphate in doubly distilled water, transferring the solution into a 100-ml volumetric flask, diluting to volume and mixing at +20 °C. Solution concentration is determined by iodometric titration. 10.00 ml of prepared solution was transferred to 100-ml volumetric flask, diluted. 10.00 ml of prepared solution was transferred into titration flask, 1 ml of 0.1 sulphuric acid solution and 1 ml of 5 % potassium iodide were added. The produced iodine was titrated with 0.02 mol/l sodium thiosulphate.

Amoxicillin (trihydrate 6-(*α-n*-oxiphenylglycylamino)-penicillanic acid), pure substance. Capsules «Gramox-A», 0.5 g produced by SP «SPERKO Ukraine» (Vinnitsa, Ukraine), series No. 081109, OSPAMOX amoxicillin tablets, 500 mg, «Sandoz GmbH», Austria.

Ampicillin trihydrate pure substance ((2S,5R,6R)-6-[(R)-2-amino-2-phenylacetyl]amino-3,3-dimetyl-7-oxo-4-tia-1-azabicyclo[3.2.0]geptan-2-carboxilate) produced by Aurobindo Pharma Ltd, India (Series No. AHT(B) 08110500, main substance content 98.8%, ω (H_2O) = 14.5%).

Oxacillin (sodium 3-phenyl-5-metyl-4-isoxazolyl-penicillin monohydrate) pure substance pharmaceutical grade produced by Aurobindo Pharma Ltd, India (Series No. OCXPD 0806002, main substance content 100.0%, ω (H_2O)=4.47%).

Ticarcillin lyophilized powder in vials for ticarcillin injection solution (2S, 5R, 6R)-6-{[(2S)-2-carboxi-2-(3-tinyl)acetyl]amino}-3,3-dimetyl-7-oxo-4-oxo-4-tio-1-azobicyclo[3.2.0] geptil-2-carboxic acid as disodium salt). *Timentin®* – lyophilized powder in vials for ticarcillin injection solution in the combined form with potassium clavulanate (ticarcin disodium 3.0 g and potassium clavulanate 200.0 mg), Smith Kline Beecham (Great Britain), series No. 456661:

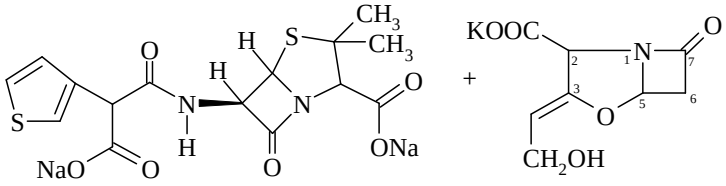


Fig.1. Structural formulas of Timentin preparation content

Experimental data were analyzed using the Excel table processor Microsoft Office Professional 2003 software package.

RESULTS AND DISCUSSION

By the method of reverse iodometric titration of $KHSO_5$ residue was determined that 1 mol of $KHSO_5$ is used per 1 mol of penicillin. The reaction finishes during 1 min and stays for 30 min (observation time at pH 3-6).

The transformation scheme of analytical determination of amoxicillin and ampicillin is given on fig.2:

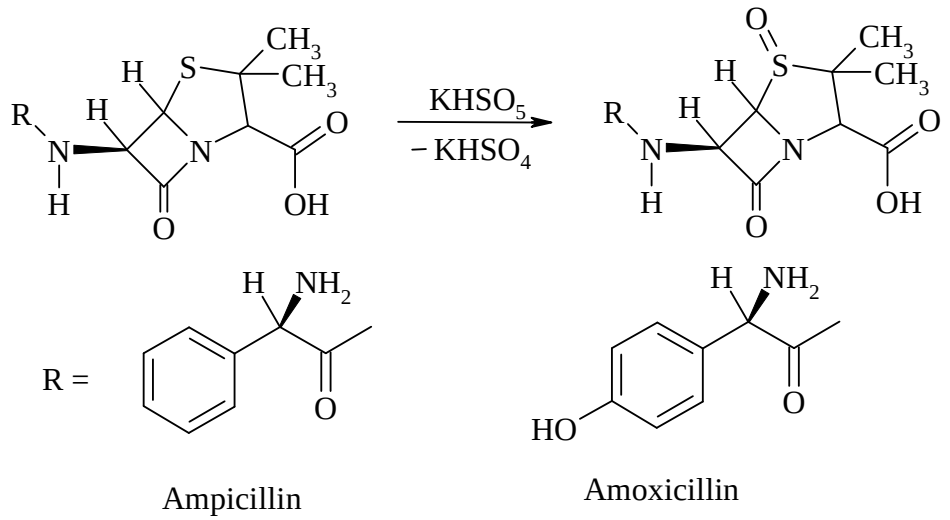


Fig.2. Scheme of penicillin S-oxidation by means of potassium hydrogenperoxomonosulphate

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

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

Amoxicillin capsules, 0.5 g assay procedure. About 0.2 g (precise weight) of capsules content was transferred to 100-ml volumetric flask diluted at weak heating by mixer containing 8 ml of dimethylformamide and 75 ml of water and brought to the mark by distilled water. 10.00 ml of prepared amoxicillin solution using pipette was transferred to 100-ml volumetric flask, 10.00 ml of 0.02 mol/l $KHSO_5$ solution was added, diluted to volume at +20°C and mixed. 10.00 ml of prepared solution was transferred into 100 ml volumetric flask, 1 ml of 0.1 mol/l sulphuric acid solution and 1 ml of 5 % potassium iodide were added. The formed of iodine was titrated with 0.02 mol/l sodium thiosulphate by means of 10 ml microburette. Blank determination was performed.

Amoxicillin content in one capsule, mg, was calculated using the equation:

$$X = \frac{0.02 \cdot K \cdot 365.4 \cdot (V_0 - V) \cdot 100 \cdot \overline{m} \cdot 100}{m_w \cdot 10 \cdot 10 \cdot 2},$$

where V_0 – sodium thiosulpahte volume used for titration in blank determination, ml;

V – sodium thiosulpahte volume used for titration in procedure, ml;

365.4 – amoxicillin (anhydrous) molar mass, g/mol;

K – correction factor of 0.0200 mol/l thiosulphate solution concentration;
$\overline{m}$ –capsule average mass, g;

m_w – weight mass, g.

Capsule analysis results are given in Table 1. Relative standard deviation is less than 2.35 % (P=0.95, n=7).

Table 1. Results of quantitative amoxicillin capsules determination by iodometric titration method using potassium hydrogenperoxomonosulphate (*P*=0.95, *n*=7)

Nominal amoxicillin mass, mg	Actual		Metrological characteristics
	mg	%	
"GRAMOX-A" capsules, 500 mg produced by SP "SPERKO Ukraine", Vinnitsa, Ukraine			
496.0 *(series 081109)	484.6 505.6 516.2 495.1 495.1 505.6 484.6	96.92 101.12 103.24 99.02 99.02 101.12 96.92	$\overline{X}$ = 498.1 (99.62%) S = ±11.71 S_x = ±4.43 $\Delta\overline{x}$ = ±10.84 *RSD* = ±2.35% ε = 2.2% δ* = +0.4 %

** An accurate amoxicillin contents by certificate (determined by BPh, 2009 [24])*

Amoxicillin tablets, 500 mg assay procedure. About 0.67 g (precise weight) of pulverized tablets powder content was transferred to 100-ml volumetric flask diluted at weak heating by mixer containing 8 ml of dimethylformamide and 75 ml of water and brought to the mark by distilled water, filtrate through glass filter No.3 into 100-ml volumetric flask, precipitate was washed by three portions by 15 ml of a mixture with the same content and brought to the mark by distilled water. Than the same operations as in quantitative determination of amoxicillin capsules were repeated.

Amoxicillin content in 1 tablet in mg was calculated using the equation as in quantitative determination of amoxicillin capsules, where $\overline{m}$ –tablet average mass, g; m_w – weight mass, g.

Amoxicillin tablets analysis results are given in Table 2. Relative standard deviation is less than 1.8 %. The procedure is available while determination of uniform mass per unit dose substance.

Table 2. Results of quantitative amoxicillin tablets determination by iodometric titration using potassium hydrogenperoxomonosulphate (*P*=0.95, *n*=7)

Nominal amoxicillin mass, mg	Actual		Metrological characteristics
	mg	%	
OSPAMOX amoxicillin tablets, 500 mg, «Sandoz GmbH», Austria			
492.0 *(ser. 158399) (475…525 mg/tablet.)	485.4 500.6 511.2 492.2 495.1 505.3 489.6	97.08 100.12 102.24 98.44 99.02 101.06 97.92	$\overline{X}$ = 497.1 (99.41%) S = 9.12 S_x = 3.45 $\Delta\overline{x}$ = 8.45 *RSD* = 1.8% ε = 1.7% δ* = +1.0 %

** An accurate amoxicillin tablets contents by certificate (determined by BPh, 2009 [24])*

Ampicillin trihydrate pure substance assay procedure. About 0.45 g (precise weight) of ampicillin trihydrate pure substance was transferred to 100-ml volumetric flask diluted at weak heating by mixer containing 8 ml of dimethylformamide and 75 ml of water and brought to the mark by distilled water at 20°C. 10.00 ml of prepared ampicillin solution using pipette was transferred to 100-ml volumetric flask, 10.00 ml of 0.02 mol/l $KHSO_5$ solution was added, diluted to volume at 20°C and mixed. 10.00 ml of prepared solution was transferred into 100 ml volumetric flask, 1 ml of 0.1 mol/l sulphuric acid solution and 2 ml of 5 % potassium iodide were added. The produced iodine was titrated with 0.02 mol/l sodium thiosulphate standard solution by means of 10 ml microburette. Blank determination was performed.

Ampicillin trihydrate pure substance content, (X, %), was calculated using the equation:

$$X = \frac{0.02 \cdot K \cdot 349.40 \cdot (V_0 - V) \cdot 100 \cdot 100\%}{2 \cdot 1000 \cdot m_w \cdot (100 - w_{H_2O})},$$

where V_0 – sodium thiosulpahte volume used for titration in blank determination, ml;

V – sodium thiosulpahte volume used for titration in procedure, ml;

349.40– ampicillin (anhydrous) molar mass, g/mol;

K – correction factor of 0.0200 mol/l thiosulphate solution concentration;

m_w – weight mass, g.

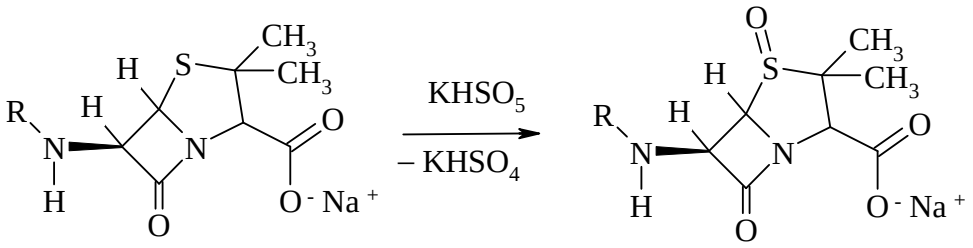
$w(H_2O)$ – water content, %.

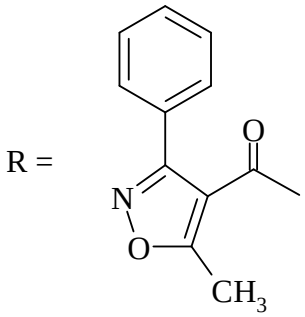
Relative standard deviation is less than 1.5 % (δ=+0.6%), (P=0.95, *n*=5). The developed procedure allows to determine pure substance assay (purity control) without standard samples. Ampicillin trihydrate pure substance results obtained by the new procedure are given in the table 3.

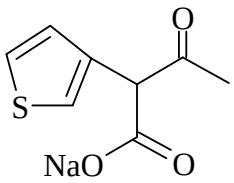
Table 3. Results of quantitative ampicillin trihydrate pure substance determination by means of potassium hydrogenperoxomonosulphate (*P*=0.95, *n*=5)

Nominal ampicillin mass, g	Actual, %	Metrological characteristics
Ampicillin trihydrate pure substance, Aurobindo Pharma Ltd, India		
0.4336 * (ser. ANT(V) 08110500)	97.82 99.79 98.80 99.80 101.81	$\overline{w}$ = 99.40 % S = ±1.51 S_x = ±0.68 $\Delta\overline{x}$ = ±1.88 *RSD* = ±1.5%; ε = 1.9% δ* = +0.6 %

** An accurate content by certificate (determined by BPh, 2009 [24])*







Oxacillin Ticarcillin

Oxacillin assay procedure. About 0.45 g (precise weight) of oxacillin substance was transferred to 100-ml volumetric flask diluted at weak heating by mixer containing 8 ml of dimethylformamide and 75 ml of water and brought to the mark by distilled water at 20°C. 10.00 ml of prepared oxacillin solution using pipette was transferred to 100-ml volumetric flask, 10.00 ml of 0.02 mol/l $KHSO_5$ solution was added, diluted to volume at 20°C and mixed. 10.00 ml of prepared solution was transferred into 100 ml volumetric flask, 1 ml of 0.1 mol/l sulphuric acid solution and 2 ml of 5 % potassium iodide were added. The formed iodine was titrated with 0.02 mol/l sodium tiosulphate standard solution by means of 10 ml microburette. Blank determination was performed.
Oxacillin sodium pure substance content, (X, %), was calculated using the same equation as in ampicillin trihydrate pure substance determination, using oxacillin (anhydrous) molar mass that equals 423.43 g/mol.

Relative standard deviation is less than 1.2 % (δ=+0.4%), (P=0.95, *n*=5). The developed procedure allows to determine pure substance assay (purity control) without standard samples.

Table 4. Results of quantitative oxacillin pure substance determination by means of potassium hydrogenperoxomonosulphate (*P*=0.95, *n*=5)

Nominal oxacillin mass, g	Actual, %	Metrological characteristics
Oxacillin sodium pure substance, Aurobindo Pharma Ltd, India		
0.4432 * (ser. OCX PD0806002)	99.01 101.00 102.02 99.01 101.00	$\overline{W}$ = 100.40 % *S* = ±1.20 S_x = ±0.54 $\Delta\overline{X}$ = ±1.49 *RSD* = ±1.2% ε = 1.5%; δ* = +0.4 %

** An accurate content as in certificate (determined by BPh, 2009 [24])*

Ticarcillin assay procedure in Timetin preparation. About 0.2 g (precise weight) pre-dried in vacuum during 30 min of vials content was transferred to 100-ml volumetric flask diluted at weak heating by mixer containing 8 ml of dimethylformamide and 75 ml of water and brought to the mark by distilled water. 10.00 ml of prepared amoxicillin solution using pipette was transferred to 100-ml volumetric flask, 10.00 ml of 0.02 mol/l $KHSO_5$ solution was added, diluted to volume at +20°C and mixed. After 2 min 10.00 ml of repapered solution was transferred into 100 ml volumetric flask, 1 ml of 0.1 mol/l sulphuric acid solution and 1 ml of 5 % potassium iodide were added. The formed iodine was titrated with 0.02 mol/l sodium thiosulphate by means of 10 ml microburette. Blank determination was performed.

Ticarcillin content in acidic form in one vial, g, was calculated using the equation:

$$X= \frac{0.02 \cdot K \cdot 384.43 \cdot (V_0 - V) \cdot 100 \cdot \overline{m} \cdot 100}{m_w \cdot 10 \cdot 10 \cdot 2 \cdot 1000},$$

where 384.43 – ticarcillin (acid) molar mass, g/mol; other values as in amoxicillin preparation analysis.

Timentin® dosage form analysis results are given in Table 5. Relative standard deviation is less than 1.68 % (P=0.95, *n*=7).

Table 5. Results of quantitative ticarcillin in Timetin® dosage form determination by means of potassium hydrogenperoxomonosulphate (*P*=0.95, *n*=7)

Nominal ticarcillin mass, g	Actual		Metrological characteristics
	g	%	
TIMENTIN® Smith Kline Beecham (Great Britain)			
3.001*(series No. 456661.)	3.0160 3.0763 2.9557 2.9858 2.9557	100.5 102.54 98.52 99.53 989.52	$\overline{X}$ = 2.9979 (99.93%) *S* = ±0.0051 S_x = ±0.0226 $\Delta\overline{X}$ = ±0.0627 *RSD* = ±1.68 ε = 2.09% δ = –0.10 %

**As given in the certificate GlaxoSmithKline (determined by BPh, 2009 [24])*

CONCLUSION

Kinetics and S-oxidation reaction of semisynthetic penicillins by means of potassium hydrogenperoxomonosulphate in aqueous solutions at pH 3-6 was studied using iodometric titration. 1 mol of any penicllin is per 1 mol of $KHSO_5$, quantitative interaction proceeds in 1 minute (observation time). The unified procedure was developed and ability of quantitative determination of penicillin by iodometric method using potassium hydrogenperoxomonosulphate ($KHSO_5$) as analytical reagent was shown. RSD ≤ 2. 35 % (δ = – 0.1…+1.0%).

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