RESEARCH ARTICLE

Quantitative Determination of *Azlocillin* by Iodometric Method using Potassium Peroxomonosulfate

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Abstract

Objective: Kinetics and stoichiometry S-oxidation reaction of sodium *Azlocillin* (Azl) by means of potassium hydrogenperoxomonosulfate in aqueous solutions using iodometric titration were studied. **Materials and Methods:** Securopen® - powder Azl sodium in flacons for preparation of solution for injections (Azl 1.0 g; 5.0 g) was used for analysis. A new iodometric method for quantitative determination of sodium Azl in Securopen® preparation using potassium hydrogenperoxomonosulfate ($KHSO_5$) as analytical reagent was proposed. Peroxomonosulfate acid as triple potassium salt $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$ (Oxone®) of "extra pure" qualification was used as oxidant. **Results:** At pH 2–4 for 1 mole of penicillin, 1 mole of $KHSO_5$ is consumed; the quantitative interaction is achieved within a time of more than 1 minute (observation time). The results were obtained by the recommended procedure for seven replicate titrations of mixtures containing the three species at various concentrations. Relative standard deviation = (0.8 −2.8) %, δ = (+0.2 …−0.31) %. It can be seen that Azl can be determined successively with good accuracy and reproducibility. **Conclusion:** The new procedure was developed and ability of quantitative determination of penicillin in pharmaceutical preparation Securopen® by iodometric method using potassium hydrogenperoxomonosulfate ($KHSO_5$) as analytical reagent was shown.

Key words: *Azlocillin*, iodometric method, pharmaceutical preparation, potassium hydrogenperoxomonosulfate, validation

INTRODUCTION

Azlocillin (Azl) is an acylampicillin antibiotic with an extended spectrum of activity and greater *in vitro* potency than the carboxypenicillins. Azl is similar to mezlocillin and piperacillin. It demonstrates antibacterial activity against a broad spectrum of bacteria, including *Pseudomonas aeruginosa* and, in contrast to most cephalosporins, exhibits activity against enterococci. By the chemical structure, penicillins are medicinal substances that belong to derivate of 6-aminopenicillanic acid (6-APA). 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]

Azl (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-{[(2*R*)-2-{[(2-oxoimidazolidin-1-yl)carbonyl]amino}-2-phenylacetyl]amino}-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid belongs to the ureidopenicillin class, and it is used for the treatment of serious infections caused by susceptible strains of microorganisms.[2]

Literature review revealed enormous analytical method was reported for the estimation of Azl individually or in combination with other drugs.

Classical iodometry of hydrolysis products is determined to be a basic method of penicillin summary quantitative determination. Its 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 does not proceed strictly

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Received: 14-12-2017
Revised: 21-03-2018
Accepted: 18-04-2018

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]

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.[4]

According to State Pharmacopoeia of Ukraine and European Pharmacopoeia,penicillin quantitative determination is performed by high-performance liquid chromatography.[5,6]

The following quantitative procedures of penicillin determination are described: Using potentiometry titration and ionometry,[7] spectrophotometry,[8-10] extraction photometry,[11] voltammetry[12] and polarography,[13] micelle electrokinetic capillary[14] and paper chromatography,[15,16] and chemiluminescence[17] and kinetic analysis methods.[18]

A new procedure for the quantitative determination of Azl sodium in the Securopen® preparation by the method of back iodometric titration using potassium hydrogenperoxomonosulfate ($KHSO_5$) as an analytical reagent was developed.

MATERIALS AND METHODS

Peroxomonosulfate 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 4.5% (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.[19]

0.1 mol/l standard sodium thiosulfate solution was prepared using the standard titer fixanal ampoule on the double-distilled water. Titrated 0.02 mol/l thiosulfate 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.

Solution of potassium iodide (5%) was prepared by dissolving 5.0 g of potassium iodine in just boiled distilled water transferring the solution into a 100-ml volumetric flask, diluting to volume and mixing.

Standard sulfuric acid solution was prepared using the standard titer fixanal ampoule on the double-distilled water $c(H_2SO_4)$ = 0.1 mol/l.

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

Solution of potassium hydrogenperoxomonosulfate (0.02 mol/l) in water was prepared by dissolving 0.615 g of potassium hydrogenperoxomonosulfate in double-distilled water, transferring the solution into a 100-ml volumetric measuring 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 measuring flask, diluted. 10.00 ml of prepared solution was transferred into titration flask, 1 ml of 0.1 sulfuric acid solution and 1 ml of 1% potassium iodide were added. The excess of iodine was titrated with 0.02 mol/l sodium thiosulfate.

Azl sodium salt substance (CAS number 37091-65-9) was used in the experiment. Azl sodium is the sodium salt form of Azl, a semisynthetic, extended-spectrum acylampicillin with antibacterial activity. Azl binds to penicillin-binding proteins located inside the bacterial cell wall, thereby inhibiting the cross-linkage of peptidoglycans, which are critical components of the bacterial cell wall. This prevents proper bacterial cell wall synthesis, thereby results in the weakening of the bacterial cell wall and eventually leading to cell lysis. Its chemical structure is following (2S,5R,6R)-3,3-dimethyl7-oxo-6-{[(2R)-2-{[(2-oxoimidazolidin-1-yl)carbonyl] amino}-2-phenylacetyl]amino}-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylate ($C_{20}H_{22}N_5NaO_6S$).

Securopen® - powder Azl sodium in flacons for preparation of solution for injections (Azl 1.0 g; 5.0 g). Manufacturer Bayer Aktiengesellschaft (245284). D-51368 Leverkusen, Germany was studied in the presented work as a medical preparation.

The procedure of preparation Azl sodium standard solution is following. 0.48109 g Azl sodium salt substance was transferred to 100-ml measuring flask, dissolve in 50 ml of double-distilled water and to bring the final volume of solution to the mark by double-distilled water.

RESULTS AND DISCUSSION

By the method of back iodometric titration of KHSO5 residue was found that 1 mol of $KHSO_5$ is consumed per 1 mol of penicillin. The reaction finishes during 1 min and stays for 30 min (observation time at pH 2–4). The transformation scheme of analytical determination of Azl is given in Figure 1.

$KHSO_5+2HCl+2KI=KHSO_4+2KCl+I_2+H_2O$

HSO +2H++3I =HSO +I +H2O

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

Kinetic dependence of light absorbance of penicillin solutions in time that have the view of curves with saturation with linear parts on the initial reaction stage are given in Figure 2.

Table 1 summarizes the results obtained by the recommended procedure for seven replicate titrations of mixtures containing

Table 1: Determination of Azl by means iodometric method using $KHSO_5$

Taken mg	Determined by kinetic method,* $\bar{X}\pm\Delta\bar{X}$	RSD (%)	$\delta=\frac{x-a}{a}\times100\%$	Recovery kinetic method (%)
1.352	1.35±0.037	2.96	0.15	99.85
2.708	2.71±0.041	1.63	0.07	100.07
5.383	5.39±0.044	0.88	0.13	100.13

*Average of seven determinations (*P*=0.95). RSD: Relative standard deviation

Table 2: Results of quantitative determination of Azl in Securopen® dosage form by means of potassium hydrogenperoxomonosulfate (*P*=0.95, n=7)

Nominal Azl mass, g	Actual g (%)	Metrological characteristics
Securopen® Bayer (Germany)		
1.001*	1.0509 (104.98)	$\bar{\chi}$= 1.003 (100.20%)
	1.0033 (100.23)	*S* = ±0.0282
	0.9954 (99.44)	$S_{\bar{\chi}}$= ±0.0107
	1.0029 (100.19)	$\Delta\bar{\chi}$= ±0.0261
	0.9962 (99.52)	RSD=2.81%
	1.0149 (101.39)	ε = ±2.60%
	0.9974 (99.64)	δ = +0.20%
5.002*	5.0150 (100.26)	$\bar{\chi}$= 4.9866 (99.69%)
	4.9012 (97.99)	*S* = ± 0.04075
	5.0135 (100.23)	$S_{\bar{\chi}}$= ±0.0154
	5.0083 (100.13)	$\Delta\bar{\chi}$= ±0.0377
	4.9771 (99.50)	RSD=0.82%
	5.0100 (100.16)	ε = ±0.76%
	4.9811 (99.58)	δ = −0.31%

*As given in the certificate Bayer (determined by BPh, 2009[20])

the three species at various concentrations. It can be seen that Azl could be determined successively with good accuracy and reproducibility.

Analysis of Azl Powder

Azl sodium (ca 500 mg) was weighed accurately, dissolved in water, and diluted to 100 ml. 10.00 ml of prepared Azl solution using pipette was transferred to 100-ml volumetric flask, 20.00 ml of 0.02 mol/l $KHSO_5$ solution was added, diluted to volume at +20°C and mixed. After 2 min, 20.00 ml of repapered solution was transferred into 100 ml volumetric flask, 2 ml of 0.1 mol/l sulfuric acid solution and 2 ml of 5 % potassium iodide were added. The excess of iodine was titrated with 0.02 mol/l sodium thiosulfate by means of 10 ml microburette. Blank determination was performed.

Azl content in acidic form ($C_{23}H_{27}N_5O_7S$) in one flacon, X g, was calculated using the equation:

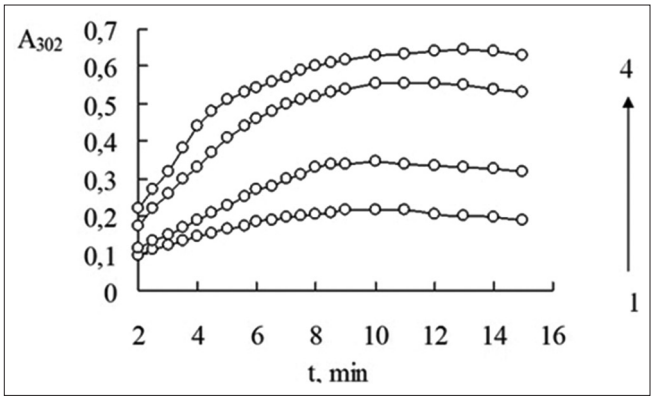

Figure 1: Effect of *Azlocillin* on the perhydrolysis reaction kinetics

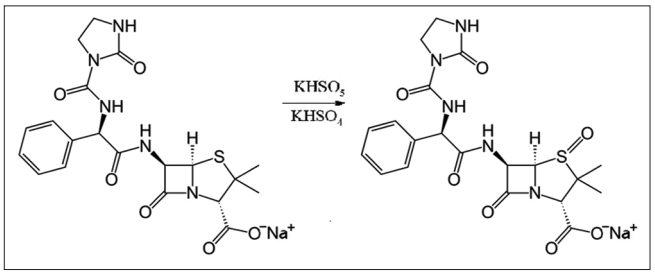

Figure 2: Scheme of *Azlocillin* S-oxidation by means of potassium hydrogenperoxomonosulfate

$$X=\frac{0.02\cdot K\cdot 461.491\cdot(V_0-V)\cdot100\cdot\bar{m}\cdot100}{m_w\cdot20\cdot20\cdot2},$$

Where, V_0 - sodium thiosulfate volume used for titration in blank determination, ml; V - sodium thiosulfate volume used for titration in procedure, ml; 461.491 - Azl (anhydrous) molar mass, g/mol; K - correction factor of 0.0200 mol/l thiosulfate solution concentration; $\bar{m}$ - flacon average mass, g; and m_w - weight mass, g.

Securopen® dosage form analysis results are given in Table 2 (P = 0.95, n = 7).

CONCLUSION

Kinetics and stoichiometry of S-oxidation reaction of sodium Azl by means of potassium hydrogenperoxomonosulfate in aqueous solutions at pH 2–4 using iodometric titration

method were studied. For 1 mole of penicillin, 1 mole of $KHSO_5$ is consumed, the quantitative interaction is achieved within a time of more than 1 min (observation time). The new procedure was developed and ability of quantitative determination of penicillin in pharmaceutical preparation Securopen® by iodometric method using potassium hydrogenperoxomonosulfate ($KHSO_5$) as analytical reagent was shown. Relative standard deviation = (0.8 −2.8) %, δ = (+0.2…−0.31) %.

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Source of Support: The research work performed in the manuscript is done on the funding of National University of Pharmacy (Ukraine). Conflict of Interest: None declared.