ORIGINAL ARTICLE

Development and Validation of the Spectrophotometric Method for the Quantitative Determination of Azlocillin in Pure Substance and Medical Preparation

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Abstract

Objective: The kinetic of conjugated reactions of S-oxidation and perhydrolysis of Azlocillin (Azl) with potassium caroate in alkaline medium is studied by the increase of forming product light absorbance at 275 nm. **Materials and Methods:** Securopen® - powder Azl sodium in flacons for the preparation of solution for injections (Azl 1.0 g and 5.0 g) was used for analysis. Peroxymonosulfate acid as triple potassium salt $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$ (Oxone®) of "extra pure" qualification was used as oxidant. A scheme of peroxyacid oxidation and perhydrolysis conjugated reactions of Azl by means of potassium caroate is proposed. A new spectrophotometric method for the quantitative determination of sodium *Azl* in Securopen® preparation using potassium caroate ($KHSO_5$) as an analytical reagent was proposed. **Results:** At pH 2–3 for 1 mole of penicillin, 1 mole of $KHSO_5$ is consumed, and the quantitative interaction is achieved within a time of more than 1 min (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 (RSD) = (0.96–2.02) %, δ = (+0.49 … – 0.33)%. It can be seen that Azl can be determined successively with good accuracy and reproducibility. **Conclusions:** The new procedure was developed and the ability of quantitative determination of penicillin in pharmaceutical preparation Securopen® by spectrophotometric method using potassium caroate ($KHSO_5$) as analytical reagent was shown.

Key words: Azlocillin, kinetics, oxidation, potassium caroate, validation

INTRODUCTION

The term antibiotic refers to a very diverse range of chemical substances that possess antibacterial activity. They can be either broad spectrum or narrow spectrum.[1] *β*-lactam antibiotics (BLAs) constitute one of the most widely used antimicrobial drugs in veterinary medicine, especially to treat and prevent bacterial infections (respiratory, urinary, and mammary gland or skin infections) of dairy cattle. This group of antibiotics can be classified into several groups according to their structural characteristics: Penicillins, cephalosporins, and more recently, carbapenems. Their unique structural feature is the presence of the four-membered BLAs (2-azetidinone) ring.[2]

Antibiotics are often used in clinical *in vitro* tests known as antimicrobial susceptibility tests (ASTs) to determine their efficacy against certain bacterial species. They are tested against Gram-negative and Gram-positive bacteria using panels, discs, and minimum inhibitory concentration (MIC) strips by medical microbiologists. ASTs decrease the risk of using an antibiotic against bacteria exhibiting resistance to it, and the results are used in clinical settings to determine which antibiotic(s) to prescribe for various infections.[3]

β-lactams inhibit the formation of peptidoglycan cross-links within bacterial cell walls by targeting penicillin-binding proteins (PBPs). Consequently, the bacterial cell wall becomes weak and cytolysis occurs. Resistance to BLAs occurs in the presence of cells containing plasmid-encoded extended-spectrum β-lactamases.

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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. Azl is considered a broad-spectrum antibiotic and can be used against a number of Gram-positive and Gram-negative bacteria. The following represents MIC susceptibility data for a few medically significant organisms.[4] The activity of Azl and mezlocillin, new semisynthetic ureido penicillins, was investigated and compared with that of other known BLAs. At a concentration of 25 µg/ml, Azlocillin (Azl) inhibited 74% of *Enterobacter*, 97% of *Proteus mirabilis*, 64% of *Citrobacter*, 91% of *P. aeruginosa*, and 82% of *Bacteroides* strains tested. Mezlocillin inhibited 86% of *Shigella*, 96% of *Enterobacter*, 80% of indole-positive *Proteus*, 88% of *Bacteroides*, and 63% of *Pseudomonas* strains tested. Azl was more active against *Pseudomonas* than ticarcillin, carbenicillin, or mezlocillin. Mezlocillin was more active than carbenicillin and ampicillin against *Escherichia coli, Klebsiella, Enterobacter, Citrobacter, Acinetobacter, Serratia*, and *Bacteroides*. Azl and mezlocillin were less active than cefazolin against β-lactamase-producing *E. coli* and *Klebsiella* strains but more active than cefazolin against *Enterobacter*, indole-positive *Proteus, Acinetobacter, Citrobacter*, and *Serratia* strains. Both compounds showed activity equivalent to that of cefoxitin against *Bacteroides* isolates. Both agents were destroyed by many of the β-lactamases from Gram-negative organisms.[5] Azl is an extended-spectrum BLAs derived from ampicillin. It is useful against *P. aeruginosa*, *E. coli*, *H. influenzae*, and a variety of other Gram-negative species. It has also been used to develop antibiotic counting techniques. Azl sodium is freely soluble in aqueous solution.[6]

Azl is only found in individuals that have used or taken this drug. It is a semisynthetic ampicillin-derived acylureido penicillin. By binding to specific PBPs located inside the bacterial cell wall, Azl inhibits the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that Azl interferes with an autolysin inhibitor.[7]

IUPAC name Azl: sodium;(2S,5R,6R)-3,3-dimethyl-7-oxo-6-[[(2R)-2-[(2-oxoimidazolidine-1-carbonyl)amino]-2-phenylacetyl]amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate. It is an antibiotic used in treating infections caused by *P. aeruginosa*, *E. coli*, and *Haemophilus* influenza.[8]

Microbiological assays on antibiotics are used in pharmaceutical preparations and raw materials whose contents are related to their biological activity and usually cannot be determined by chemical analyses.[9]

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.[10]

The extensive literature survey reveals the various methods of quantitative determination of penicillin family preparations, such as high-performance liquid chromatography, spectrophotometry, extraction photometry, iodometry, different variants of voltammetry, electrokinetic capillary chromatography, and densitometry, and kinetic analysis is proposed.[11-16]

The spectrophotometric methods that are based on the application of phenol Folin–Ciocalteu reagent and reactions with Mn(II), Cu(II), and Ni(II) salts are also known. These methods give the possibility to determine penicillin in medical preparations in the presence of different excipients.[17-21]

The β-lactam group is one of the most important families of antibiotics used in veterinary medicine and has been widely used for decades in animal husbandry. Penicillins are commonly used antimicrobials for the treating, controlling, and preventing of animal diseases. The extensive use of penicillins may cause the presence of their residues in food products of animal origin and may have side effects to consumers. Moreover, penicillin residues in food products may be responsible for allergic reactions in humans and promote the occurrence of antibiotic-resistant bacteria. Therefore, rapid and sensitive methods are needed for the routine to monitor the presence of residues in animal tissues.

It is based on the preliminary oxidation of Azl with potassium caroate excess to the corresponding S-oxide, followed by the determination of the hydrolytic conversion of its product in an alkaline medium by the kinetic spectrophotometric method (initial rate [tangent] method).[22]

MATERIALS AND METHODS

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

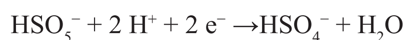
Azl sodium salt substance (CAS Number 37091-65-9) was used in the experiment. Azl sodium is the sodium salt form of Azl, a semisynthetic, extended-spectrum acylampicillin with antibacterial activity. Azl binds to PBPs located inside the bacterial cell wall, thereby inhibiting the cross-linkage of peptidoglycans, 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 leads to cell lysis. Its chemical structure is

following (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-carboxylate ($C_{20}H_{22}N_5NaO_6S$).[1]

Investigated pharmaceutical preparation Securopen® - powder Azl sodium in flacons for preparation of solution for injections (Azl 1,0 g; 5,0g). Manufacturer Bayer Aktiengesellschaft (245284). D-51368 Leverkusen, Germany, was studied in the presented work as a medical preparation.

The procedure for the preparation of Azl sodium standard solution is as follows: 500 mg Azl sodium salt substance was transferred to 100-ml measuring flask and dissolved in 50 ml of double distilled water and to bring the final volume of solution to the mark by double distilled water.

Potassium caroate 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 and has good solubility and stability in water. It was proposed for cefadroxil kinetic spectrophotometric determination as an analytical reagent. Standard electrode potential for redox semi reaction



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

is 1.8 V.[23-26]

The procedure of potassium caroate standard solution is as follows: 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 potassium caroate was standardized iodometrically.

Electrochemical measurements were carried out in the spectrophotometer SF-46 (LOMO); kinetics was studied by the produced product light absorbance at 280 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 solution of sodium hydroxide (NaOH) without carbonates was used to create and maintain the required acidity. Processing of the results was carried out by "tangent method" (the differential version). Rate was estimated by the slope of the linear section of the kinetic curve A - time ($tg\alpha_{azl}$, min^{-1}).

Kinetic method have certain advantages in pharmaceutical analysis regarding selectivity and elimination of additive interferences, which affect direct spectrophotometeric method. Measurements of the pH were made on potentiometer (Gomel, Belarus, I-160 model).

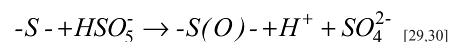
The method on initial rates (tangent method) was used to collect kinetic data (usually at 275 nm) by following the appearance production of perhydrolysis reaction of Azl. A solution of NaOH was thermostated in the cell compartment, and then, mixtures of solutions of Azl with solutions of potassium caroate (time incubation of 1 min) were added to the cell. The resulting solution was mixed thoroughly and put into the spectrophotometer. The precision of rate determination was usually ±2–5%.

Aliquots of 0.50–10.00 mol L^{-1} of the studied Azl test solutions were pipetted into a series of 50 mL volumetric flask containing 5 mL of 2.10^{-2} mol/L $KHSO_5$ solution and mixed well. 5 mL of 0.06 mol/L 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 275 nm was recorded as a function of time for 10 min against reagent blank. It shows the dependence of Azl alkaline solution absorption against time at 275 nm. They have linear dependence during the first 10–15 min. 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 Azl (C, µg/mL).

For simplicity, we use the following terminology. The potassium caroate (both HSO_5^- and SO_5^{2-}) is termed as caroate system, the perhydrolysing mixture of potassium caroate with NaOH solution will be termed the perhydrolysis system SO_5^{2-} +.

SO_5^{2-} +HO^- was considered to be the active perhydrolysant in these reactions, and the data are consistent with this interpretation.

Furthermore, a preliminary kinetic study by iodometric titration method was conducted on the S-oxidation of Azl by the potassium caroate system; the reaction is



$$-S- + HSO_5^- \rightarrow -S(O)- + H^+ + SO_4^{2-}.$$ [29,30]

A mechanism involving the intermediate S-oxide Azl (formed in a rapid step) and its conjugate with perhydrolysis system was postulated. Product perhydrolysis reaction of Azl (λ_{max} 275 nm)

Simple and sensitive kinetic spectrophotometric potassium caroate in acidic medium to form S-oxide with follow in product perhydrolysis is in strong alkaline medium at the room temperature.

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 Azl under study with potassium

caroate and therefore with alkali solution. Maximum activity of potassium caroate in the reaction was achieved at concentrations $2 \cdot 10^{-3}$ mol/L.

A structure Azl is presented in Figure 1.

The electronic spectra of interaction product of Azl with reagents depending on the time are shown in Figure 2.

The theoretical scheme of transformation of the reaction product is given in Figure 3.

The calibration plot for kinetic determination of Azl in optimum conditions is given in Figure 4 shows that the tgα linear concentration dependence was observed within the Azl content in solution 1–50 µg/mL. This was a precondition for the possibility of using the kinetic method in the analysis.

Table 1 shows the results obtained by the recommended procedure for seven replicate titrations of mixtures containing the three species at various concentrations. It can be seen that Azl could be determined successively with good accuracy and reproducibility.

The procedure of Azl assay in flacons. Azl sodium (ca 500 mg) was weighed accurately, dissolved in water, and diluted to 100 ml. 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. Got solutions Azl was measured photometrically in a quartz cuvette at 275 nm against distilled water (compensation solution) during first 15 min every minute, and the absorbance kinetic curves against time were constructed. The slope of the linear section of the kinetic curve, tgα, was determined.

The content of $C_{20}H_{22}N_5NaO_6S$, in g, in one flacon (X_{Azl}) was calculated using the following equation:

$$X_{Azl} = \frac{a_{st} . tg\alpha . \bar{a}}{a . tg\alpha_{st}}$$

where a_{st} - the sample weight of the work standard of Azl, g;

tgα_{st} - the slope ratio of the kinetic curve in the experiment with the work standard of Azl, min^{-1};

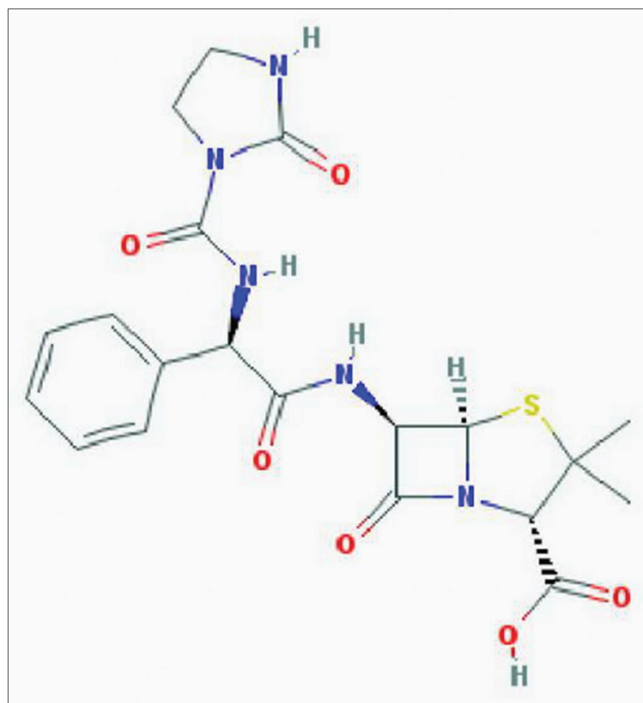


Figure 1: The structure of Azlocillin

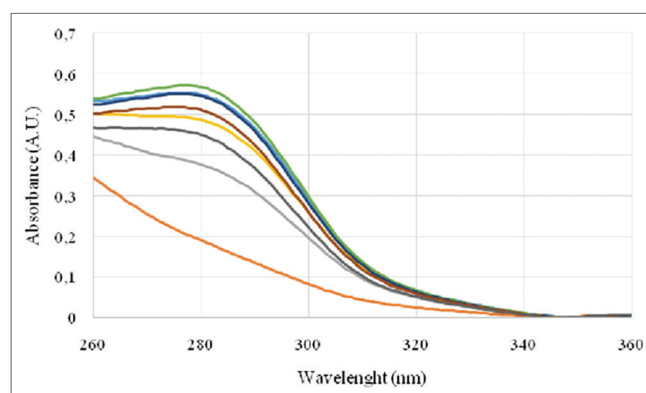


Figure 2: Ultraviolet absorption spectra of system of $2 \cdot 10^{-4}$ mol/l Azlocillin with $2 \cdot 10^{-3}$ mol/l potassium caroate in 0.01 mol/l sodium hydroxide as function of time (min): 1–3; 2–7; 3–11; 4–15; 5–19; 6–23; 7–27; 8–31

Table 1: Determination of Azl by kinetic method with use $KHSO_5$ as oxidizing agent

Taken g	Determined by kinetic method,* $\bar{X} \pm \Delta\bar{X}$	RSD (%)	$\delta = \frac{x-a}{a} \times 100$	Recovery kinetic method (%)
1.333	1.35±0.028	2.81	+1.28	101.28
2.813	2.83±0.043	1.34	+ 0.60	100.60
5.541	5.56±0.051	0.68	+0.34	100.34

*average of seven determinations (*P*=0.95), RSD: Relative standard deviation

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

Nominal Azl mass, g	Actual g (%)	Metrological characteristics
SECUROPEN® Bayer (Germany)		
1.001*	1.0029 (100.19)	$\bar{X}$ =1.006 (100.50%)
	1.0138 (101.28)	S=±0,02036
	0.9818 (98.08)	$S_{\bar{X}}$ =±0.00769
	1.0397 (103.87)	$\Delta\bar{x}$=±0.01885
	1.0101 (100.91)	RSD=2.02%
	1.0129 (101.19)	ε=±1.87%
	0.9809 (97.99)	δ=+0.49%
5.002*	5.0179 (100.32)	$\bar{X}$ =4.9856 (99.67%)
	5.0035 (100.03)	S=±0.04774
	4.9015 (97.99)	$S_{\bar{X}}$ =±0.01804
	5.0098 (100.16)	$\Delta\bar{x}$=±0.044207
	4.9333 (98.63)	RSD=0.96%
	5.0159 (100.28)	ε=±0.89%
	5.0172 (100.30)	δ=–0.33%

*content of Azl in preparation was controlled by the independent method of iodometric titration.[3]RSD: Relative standard deviation, Azl: Azlocillin

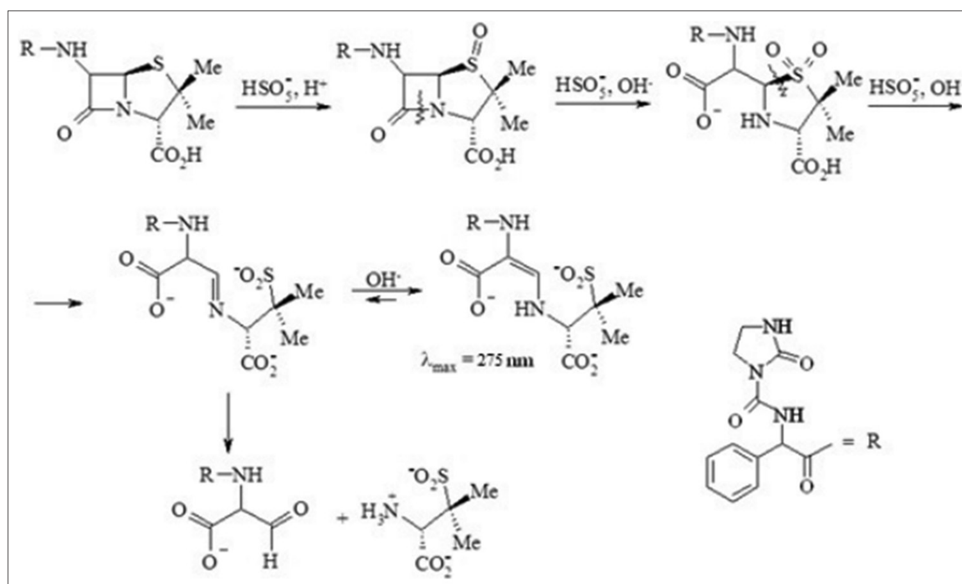


Figure 3: The scheme of peroxy acid oxidation and perhydrolysis conjugated reactions of Azlocillin

a - the sample weight of the studied powder of Azl, g;

$\bar{a}$ - the average weight of the flacon, g;

tgα - the slope ratio of the kinetic curve in the experiment with the Azl solution, min^{-1}.

The results of the Azl quantitative determination are given in Table 2. The proposed method has good accuracy, RSD = (0.96–2.02)%.

CONCLUSION

The reaction kinetics of the peroxy acidic oxidation and perhydrolysis of Azl with potassium caroate in the alkaline medium are studied. As an oxidizing agent, the potassium triple salt of peroxymonosulfuric acid, $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$, syn. “Oxone,” was applied. The procedure was developed and the possibility of the quantitative determination of Azl in the

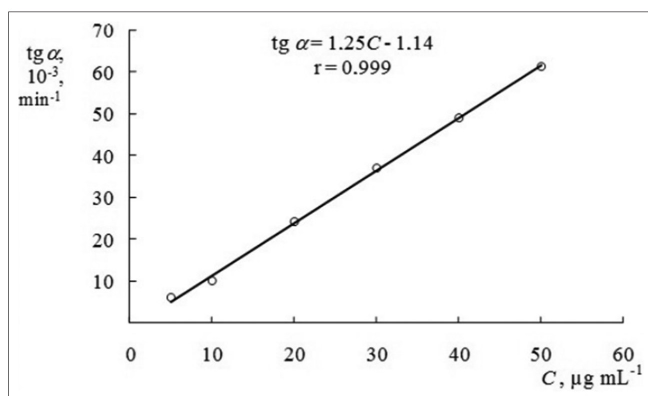


Figure 4: The calibration plot for kinetic determination of Azlocillin using potassium caroate

Securopen® preparation based on the results of the kinetic-spectrophotometric method with potassium caroate as reagent was shown. RSD=(0.96–2.02)%, δ=(+0.49 … – 0.33)%.

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Conflicting Interest (If present, give more details)

The authors have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements) or non-financial interest (such as personal or professional relationships, affiliations, and knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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