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ANALYTICAL DEVELOPMENT AND VALIDATION OF SIMPLE PROCEDURES FOR THE DETERMINATION OF MEZLOCILLIN BY TWO ALTERNATIVE METHODS

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This article addresses the search for new analytical reactions and the determination of optimal conditions for their implementation, which can serve as the basis for quantitative analysis of penicillins. Two unified procedures were developed, and the possibility of quantitative determination of mezlocillin in a pure substance and pharmaceutical formulation by kinetic spectrophotometry and redox titration using potassium caroate was demonstrated. A scheme of the chemical transformation of mezlocillin in the reaction with potassium caroate is proposed. The kinetics of the coupled reactions of S-oxidation and perhydrolysis of mezlocillin with potassium caroate in an alkaline medium were studied by monitoring the increase in absorbance of the formed product at 290 nm. The appearance of a new absorption band enabled the development of a new procedure for the quantitative determination of mezlocillin. The reaction rate was monitored spectrally and displayed in real time. A differential variation of the tangent method was used to process the kinetic data.

Keywords: antibiotic, mezlocillin, kinetic spectrophotometry, redox titration, perhydrolysis, potassium caroate.

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Introduction

Mezlocillin (Mezl) is a penicillin beta-lactam antibiotic used in the treatment of bacterial infections caused by susceptible, usually gram-positive, organisms. The name «penicillin» can either refer to several variants of penicillin available, or to the group of antibiotics derived from the penicillins. Mezl has in vitro activity against gram-positive and gram-negative aerobic and anaerobic bacteria. The bactericidal activity of Mezl results from the inhibition of cell wall synthesis and is mediated through Mezl binding to penicillin binding proteins (PBPs). Mezl is stable against hydrolysis by a variety of beta-lactamases, including penicillinases, and cephalosporinases and extended spectrum beta-lactamases [1].

By binding to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, Mezl 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 Mezl interferes with an autolysin inhibitor [2].

Penicillins, so-called beta-lactam antibiotics, are characterized by three fundamental structural requirements: a condensed beta-lactam (BLA) structure (shown in blue and red rings), a free carboxylic acid group (shown in red at lower right), and one or more

substituted amino acid side chains (shown in black). Figure 1 shows the lactam structure can also be viewed as the covalent bonding of pieces of two amino acids: cysteine (blue) and valine (red)[1].

BLAs are most commonly used to treat bacterial infections, accounting for nearly 60% of global antibiotic usage, due to their broad spectrum of antimicrobial activity. Figure 2 shows the resistance mechanisms of bacteria to BLAs are the production of β-lactamases, the modified active sites of PBPs, the down-regulation of outer-membrane proteins (OMPs), and the overexpression of efflux pumps [3].

Mezl shown in Fig. 3 is chemically known as by IUPAC Name (2S,5R,6R)-3,3-dimethyl-6-[[(2R)-2-[(3-methylsulfonyl-2-oxoimidazolidine-1-carbonyl) amino]-2-phenylacetyl] amino]-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid.

The technology for the preparation of Mezl includes the following steps: ampicillin trihydrate and 1-chloroformyl-3-methylsulfonyl-2-imidazolidinone are subjected to an acylation reaction in alkaline conditions, and then extraction, acidification and crystallization are carried out to obtain Mezl. Figure 4 shows the synthesis scheme of Mezl.

Mezlocillin is a white to pale yellow crystalline powder. Mezl is freely soluble in water. The pH of a 10% aqueous solution is between 4.5 and 8.0. Chemical formula $C_{21}H_{25}N_5O_8S_2$. Molecular weight 539.6 g/mol.

The quantitative determination of drugs penicillin series becomes more and more important. The control of the quality and quantity is one of the obligatory steps for manufacturing medicines. The number of medicines produced increases from year to year and the quality of the drugs has to be controlled. Therefore,

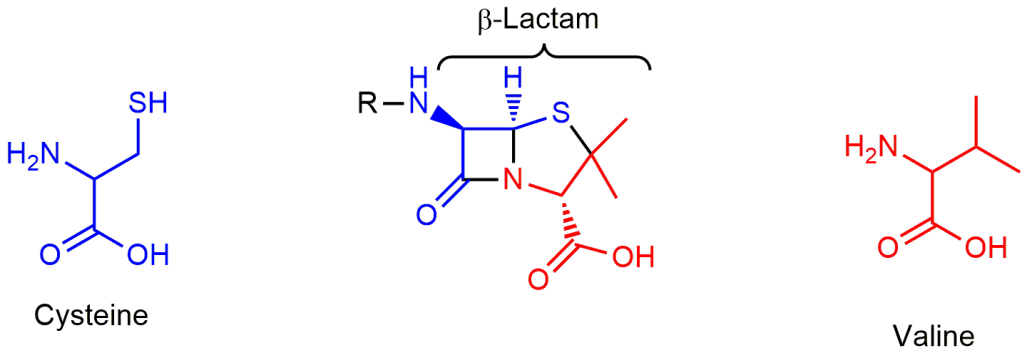

Fig. 1. Beta-lactam structure

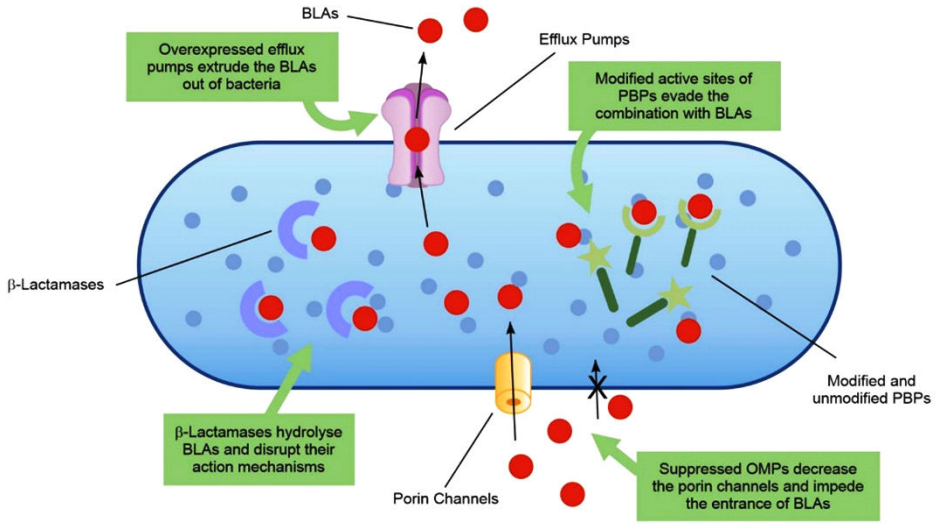

Fig. 2. Proposed bacterial mechanisms of resistance to β-lactam antibiotics (BLAs), including the production of β-lactamases, the modified active sites of penicillin-binding proteins (PBPs), and down-regulated outer membrane proteins (OMPs)

[1] United States Pharmacopeial Convention, Rockville, Maryland. 2015, 4. https://search.worldcat.org/title/The-United-States-pharmacopeia-:-the-national-formulary/oclc/933365422.

the development of new procedures that are easy to perform and cost-effective is of great interest. The procedures proposed should be unified, selective, sensitive, and precise, and they should be validated by the monograph "Validation of analytical methods" of the State Pharmacopeia of Ukraine (SPhU). European Pharmacopoeia (EPh) penicillin quantitative determination is performed by high performance liquid chromatography (HPLC). International Pharmacopoeia recommends determining the penicillin summary in semisynthetic penicillin by neutralization method after preparation hydrolysis by excess of sodium hydroxide titrated solution at heating [4–6].

The analysis of literary data shows that a promising direction of scientific research is to find out the possibility of carrying out the analysis of penicillins. The methods that are currently used to determine penicillins in pharmaceutical preparations have been reviewed. They include analytical measurement and appliance, equipment designed to perform a specific task in dependency of detection methods.

Methods of potentiometry titration, amperometry, high-performance liquid chromatography (HPLC), voltammetry, polarographic analysis, micelle electrokinetic capillary, spectrophotometry, chemiluminescense, iodometry and others [7–13] for the quantitative determination of penicillin drugs are well described in scientific articles.

The issue of quantitative determination of penicillins does not lose its relevance. Most of the known methods for the quantitative determination of penicillins are reduced to the determination of the final products of their hydrolytic cleavage, which are obtained at the previous stage of analysis. They are long-lasting and require heating.

The methods for determining Mezl developed by us have a number of advantages over the already known ones: they allow determining Mezl in much smaller quantities, do not require long-term heating of the reaction mixture simple and faster.

The developed spectrophotometric method is time saving, simple, accurate, economic, sensitive and reproducible, can be used in quality control laboratories. In addition, the principal advantages of the present method are that it is rapid and enough precise comparing with other methods of assay.

Thus, this article is devoted to the search for an analytical reaction and finding out the optimal conditions for its course, which can be used as a basis for the quantitative determination of Mezl using potassium caroate.

Experimental

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

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

is 1.81 V.

Substances and solutions

For the research, mezlocillin sodium salt of pharmacopoeial purity, a dry sterile powder in vials (1.0 g) for injection «Mezlocillin Sodium», China Pharma Supplier produced by «Ningbo Feiyue Trading

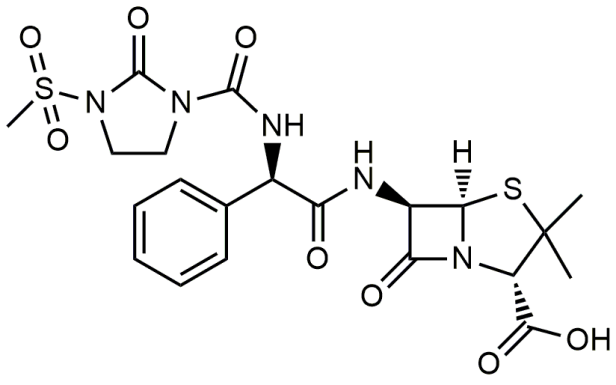

Fig. 3. Chemical structures of mezlocillin

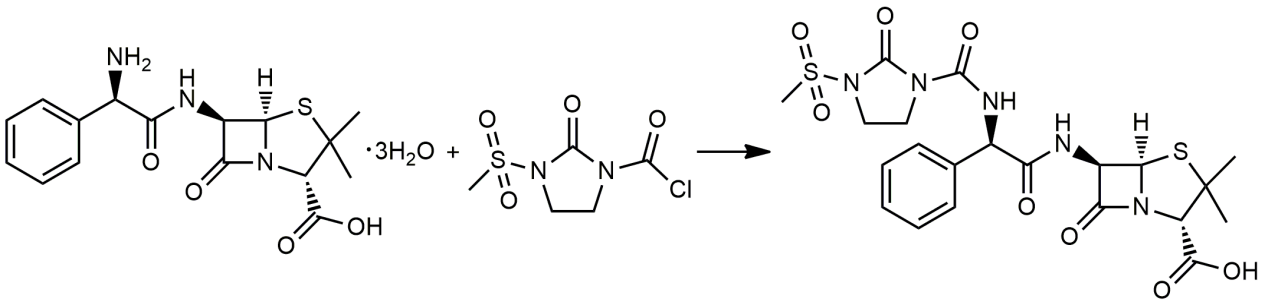

Fig. 4. MEZL synthesis

Co. Ltd» was used. Potassium caroate was obtained from commercial sources and used as an oxidant in the form of a triple potassium salt ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$, «Oxone») of «extra pure» grade with an active oxygen content of 4.5%. The choice of the reagent was due to its availability, fairly good solubility and stability in aqueous solutions, and a relatively high oxidizing ability.

Working solution of potassium caroate, $2 \cdot 10^{-2}$ mol·L^{-1}

A weighed portion of 0.6148 g of the salt was dissolved in 100.0 mL of double-distilled water at 20^{0}C. The solution concentration was controlled by iodometric titration.

As a standard sample of Mezlocillin sodium salt, we used the substance of Mezl of pharmacopoeial purity with the content of the main substance of 98.0%.

Standard sample solution of Mezl, $1 \cdot 10^{-2}$ mol·L^{-1}

0.5620 g of mezlocillin sodium was diluted in double-distilled water in a 100 mL flask at 20^{0}C.

Working solutions of Mezl

Seven aqueous solutions of the following concentrations (%): 55; 75; 100; 115; 125; 135; and 155 were prepared in 100 mL volumetric flasks; the corresponding portions of 0.2968; 0.4047; 0.5396; 0.6205; 0.6745; 0.7285; and 0.8364 of the Mezl substance were weighed (g).

Sodium thiosulfate solution, $2 \cdot 10^{-2}$ mol·L^{-1}

An ampoule of a standard titer of sodium thiosulfate with an exact concentration of 0.1 mol·L^{-1} was diluted five times with distilled water.

Solution of potassium iodide, 5%

A weighed portion of 5.0 g of potassium iodide was dissolved in 50 mL of distilled water, and the solution was diluted to the volume in a 100 mL volumetric flask at 20^{0}C.

Sodium hydroxide solution, $6.25 \cdot 10^{-2}$ mol·L^{-1}

The sodium hydroxide solution was prepared according to Hillebrant by diluting the saturated solution with freshly distilled water.

Sulfuric acid, 0.1 mol·L^{-1}

An ampoule of a standard titer of sulfuric acid with an exact concentration of 0.1 mol·L^{-1} was diluted with distilled water.

Methods

Spectrophotometry

The spectra of solutions of Mezl and its oxidation products were recorded, and the light absorption of solutions in a quartz cuvette per 1 cm was measured on an Evolution 60S UV-Visible Spectrophotometer Thermo-Scientific (USA) against the solution without Tic or double-distilled water (compensation solution).

Titration

The titer of the Mezl solution studied was determined using a 10 mL microburette with an accuracy of ±0.01 mL filled with a titrant to the zero mark.

Procedures

Kinetic spectrophotometric method

Close 50 mg (accurate weight) of the powder of the Mezl sodium salt studied was transferred into a 100 mL volumetric flask, dissolved in 50 mL of distilled water, the solution was diluted to the volume, and the content was mixed. 5.00 mL of the solution obtained was transferred into a 50 mL volumetric flask, 5.0 mL of a $2 \cdot 10^{-2}$ mol·L^{-1} $KHSO_5$ solution and 5.0 mL of NaOH with the concentration of $6.25 \cdot 10^{-2}$ mol·L^{-1} was added. The resulting solution was exposed to photometric measurements for 10 min in a 1 cm quartz cuvette at 290 nm using distilled water as a compensation solution.

Reduction-oxidation (redox) titration method

Close 950 mg (accurate weight) of the powder of the Mezl sodium salt studied was dissolved in 75 mL of water in a 100 mL volumetric flask at 20^{0}C, and diluted to the volume. Using a pipette, 10 mL of the resulting Mezl solution was taken and transferred to a 100 mL volumetric flask, 10.0 mL of a $2 \cdot 10^{-2}$ mol·L^{-1} $KHSO_5$ solution was added with stirring, and diluted to the volume with distilled water at 20^{0}C. Using a pipette, 10 mL of the reaction mixture was taken and transferred to a 100 mL flask, acidified with 1 mL of a 0.1 mol·L^{-1} H_2SO_4 solution, and 2 mL of a 5% potassium iodide solution was added with vigorous stirring. The displaced iodine was immediately titrated with a standard $2 \cdot 10^{-2}$ mol·L^{-1} sodium thiosulfate solution. In parallel, under the same conditions, a control experiment is carried out (without the Mezl solution studied).

Results and discussion

Kinetic spectrophotometric method

Effect of Caro's acid

1 mL of $1 \cdot 10^{-2}$ mol·L^{-1} solution of Mezl was pipetted into 100 mL volumetric flasks containing 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, and 7 mL of $2 \cdot 10^{-2}$ mol·L^{-1} $KHSO_5$ solution and 1 mL of $6.25 \cdot 10^{-2}$ mol·L^{-1} NaOH solution. The content of the mixture of each flask was mixed well, and the increase in absorbance at 290 nm was recorded for 30 min against the reagent blank as a function of time. It showed the dependence of absorption at 290 nm of Mezl alkaline solutions against time as a function of the acid concentration. A linear dependence was observed for the first 30 min.

The maximum slope was obtained when 5.0 mL of $2 \cdot 10^{-2}$ M Caro's acid was used. Thus,

5 mL of $2 \cdot 10^{-2}$ M Caro's acid was chosen as the optimal value.

Effect of the sodium hydroxide concentration

1 mL of $1 \cdot 10^{-2}$ mol·L^{-1} solution of Mezl was pipetted into 100 mL volumetric flasks containing 0.5 mL, 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, and 7 mL of $6.25 \cdot 10^{-2}$ mol·L^{-1} NaOH solution of $2 \cdot 10^{-2}$ mol·L^{-1} $KHSO_5$ solution. The content of the mixture of each flask was mixed well, and the increase in absorbance at 290 nm was recorded for 30 min against the reagent blank as a function of time. It showed the dependence of the absorption at 290 nm of Mezl alkaline solutions against time as a function of the acid concentration. A linear dependence was observed for the first 10–15 min.

The maximum slope was obtained when 5 mL of $6.25 \cdot 10^{-2}$ mol·L^{-1} NaOH was used. Thus, 5 mL of $6.25 \cdot 10^{-2}$ mol·L^{-1} NaOH was chosen as the optimal value.

As a result of the study, it was found that the order of mixing the solutions significantly affected the kinetics and the yield of the reaction product: the highest rate of the product formation was after the preliminary mixing of the Mezl solution with $KHSO_5$ (the stage of the Mezl sulfoxide formation).

Effect of the mezlocillin concentration

Without $KHSO_5$ under the above conditions, no reaction product was formed for 30 min. The necessary excess of $KHSO_5$ can be explained by the influence of further hydrolytic decomposition of S-oxide Mezl in the alkaline medium (nucleophilic catalysis of the hydrolysis of the β-lactam and thiazolidine cycles). Due to the alpha effect, $KHSO_5$ is a stronger nucleophile than hydroxide ion by many times (Fig. 5). PMS-induced oxidation of β-lactam antibiotics was proposed to proceed through a non-radical mechanism involving direct two-electron transfer along with the heterolytic cleavage of the PMS peroxide bond. The product analysis indicated oxidation of β-lactam antibiotics to two stereoisomeric sulfoxides [14].

Plotting a calibration graph

Using a microburette, 0.50; 2.50; 3.00; 4.00; 5.00; and 6.00 mL samples of the standard Mezl solution were added to 50 mL volumetric flasks followed by 5 mL of $2 \cdot 10^{-2}$ mol·L^{-1} $KHSO_5$ solution put to each flask, and the content was shaken thoroughly. 5.0 mL of $6.25 \cdot 10^{-2}$ mol·L^{-1} NaOH solution were sequentially poured into each flask; the solution was diluted to the volume with distilled water and thoroughly mixed. After adding alkali to the solution, the stopwatch was started. The resulting solutions were photometered in a quartz cuvette with a thickness of 1 cm at 290 nm against distilled water (compensation solution) for 10 minutes every minute at 20^{0}C, and kinetic curves of the dependence of the optical density on time were plotted. According to the slope of the linear sections of the kinetic curves, a calibration dependence of tga on the concentration of Mezl (c, μg·mL^{-1}) was constructed.

Figure 6 shows a calibration graph for determining Mezl, according to which, the dependence of concentration on tgб is linear in the range of 5 to 60 μg·mL^{-1}. This makes it possible to determine the quantitative content of Mezl in the given concentration range by the standard method.

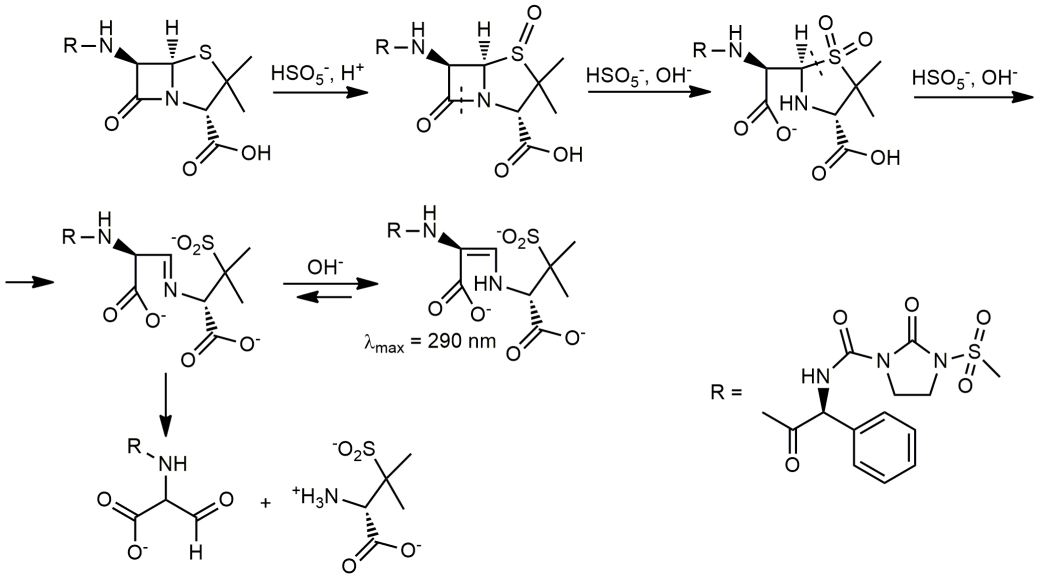

Fig. 5. Scheme of coupled reactions of peroxyacid oxidation and perhydrolysis of sulfon Mezl with the formation of a substituted derivative of N-acryl-β-penicylamine sulfate

The content of $C_{21}H_{25}N_5O_8S_2$, in mg in one vial, (X_{Mezl}) was calculated by the following formula:

$$X_{Mezl} = \frac{a_{st} \cdot tg\alpha \cdot \bar{a} \cdot w}{a \cdot tg\alpha_{st}}, \quad (1)$$

where a_{st} is the mass of a standard sample of Mezl sodium salt, mg; tga_{st} is the slope of the kinetic curve in the study with the standard solution of Mezl sodium salt, min^{-1}; w is the content of $C_{21}H_{24}N_5NaO_8S_2$ Mezl sodium salt in the standard sample of Mezl, in mass fractions; a is the weighed portion of the powder of Mezl sodium salt studied, mg; $\bar{a}$ is the average weight of the drug in the vial, mg; and tga is the slope of the kinetic curve in the study with the test solution of Mezl sodium salt, min^{-1}.

The results of the analysis of the Mezl drug by kinetic spectrophotometric method are shown in Table 1. The relative standard deviation did not exceed 0.4% (δ=−0.1%).

Redox titration method

By the method of reverse redox titration of the $KHSO_5$ excess, it was found that in the reaction studied 1 mol of $KHSO_5$ was consumed by 1 mol of Mezl, and the interaction between them occurred for 1 min. The analytical reaction underlying the method is shown in Fig. 7.

The content of $C_{21}H_{25}N_5O_8S_2$ (X, in %) was calculated by the following formula:

$$X = \frac{0.02 \cdot K \cdot 539.6 \cdot (V_0 - V) \cdot 100 \cdot 100\%}{2 \cdot 1000 \cdot m_s \cdot (100 - w_{H_2O})}, \quad (2)$$

where V_0 is the volume of sodium thiosulfate solution in the control experiment, mL; V is the volume of sodium thiosulfate solution studied, mL; 539.6 is the molar mass of mezlocillin (anhydrous), $g \cdot mol^{-1}$; K is the correction coefficient for the concentration of sodium thiosulfate solution to 0.0200 $mol \cdot L^{-1}$; and m_s

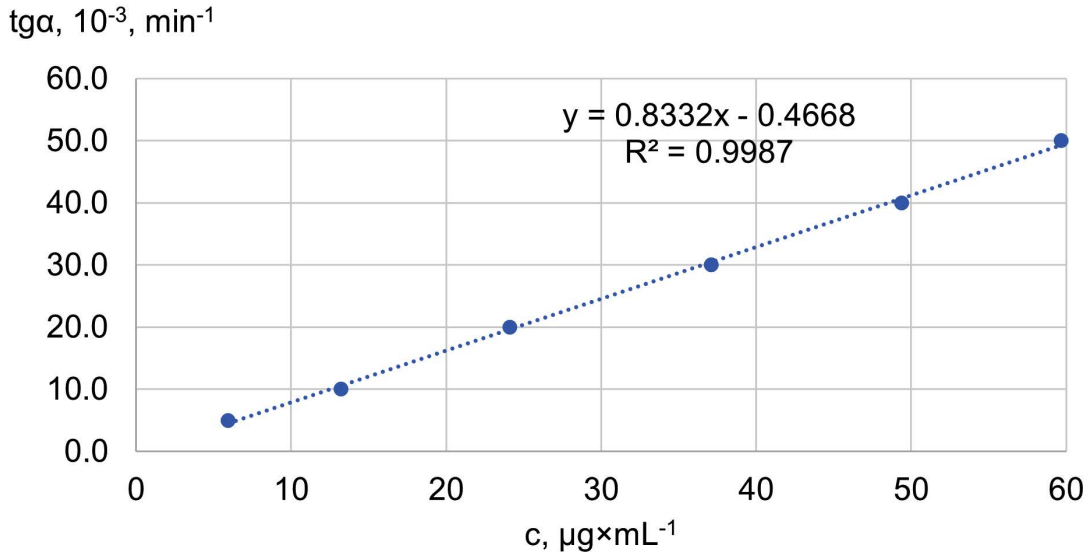

Fig. 6. Calibration graph for the quantitative determination of Mezl, c(NaOH)=6.25·10⁻² mol·L⁻¹; c($KHSO_5$)=2.0·10⁻² mol·L⁻¹

Table 1

Results of the quantitative determination of Mezlocillin by the kinetic-spectrophotometric method in the Mezl drug according to the reaction with potassium caroate (P=0.95, n=7)

Mezlocillin taken, mg	Found		Results of processing statistical data
	mg	%	
978.0[a]	981.3	98.1	$\bar{x}$=977.0 (97.7%)
	975.5	97.6	S=±3.89423
	977.6	97.8	S_x=±1.47188
	982.8	98.3	$\Delta\bar{x}$=±3.60611
	976.5	97.7	RSD=±0.40%
	973.4	97.3	ε=±0.39%
	972.2	97.2	δ[b]=–0.1%

Notes: [a] – the Mezl content indicated in the quality certificate (μ); [b] – $\delta = (\bar{x} - \mu) \cdot 100\% \cdot \mu^{-1}$.

is the weighed portions of Mezl, g.

The results of the analysis of the Mezl drug by redox titration are shown in Table 2. The relative standard deviation did not exceed 0.2% (δ=+0.62%).

Conclusions

The possibility of analytical determination of mezlocillin by the biologically active part of the molecule (alicyclic sulfur and β-lactam ring) is shown, the proposed methods give reproducible and accurate results. The developed methods have good specificity and allow determining the content of the main component of mezlocillin, avoiding the influence of impurities. The results of accuracy and precision are in good agreement with the results obtained by the reference method.

Using the methods of kinetic-spectrophotometric and redox titration, two independent procedures for the quantitative determination of mezlocillin in the substance and the drug product have been developed using potassium caroate as an analytical reagent ($KHSO_5$).

The developed methods of quantitative determination of mezlocillin can be used to develop analytical regulatory documentation for medicinal products, as well as in the practice of state laboratories for quality control of medicinal products and central factory laboratories of pharmaceutical enterprises.

The proposed methods of performing the analysis do not require the use of expensive devices, as well as toxic chemical reagents. In terms of sensitivity, speed of execution and selectivity, the developed methods of analysis are better and more economically profitable than the existing ones.

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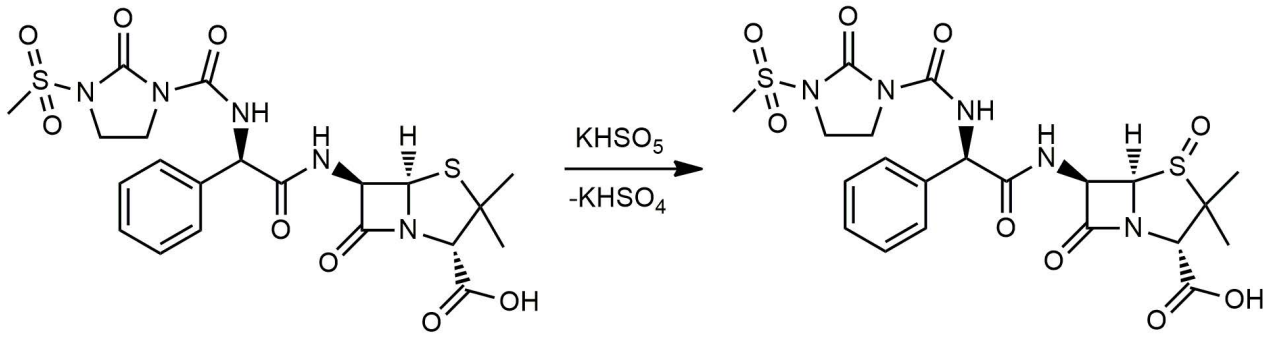

Fig. 7. Scheme of S-oxidation of Mezl by potassium caroate

Table 2

Results of the quantitative determination of mezlocillin by redox titration in the Mezl drug by the reaction with potassium caroate (P=0.95, n=7)

Mezlocillin taken, mg	Found		Results of processing statistical data
	mg	%	
978.0[a]	983.4	98.3	$\bar{x}$=984.1 (98.4%)
	985.1	98.5	S=±1.74671
	982.6	98.3	S_x=±0.66019
	986.2	98.6	$\Delta\bar{x}$=±1.61747
	981.3	98.1	RSD=±0.18 %
	985.5	98.6	ε=±0.16%
	984.7	98.5	δ^b=+0.62%

Notes: [a] – the Mezl content indicated in the quality certificate (μ); [b] – $\delta = \left(\bar{x} - \mu\right) \cdot 100\% \cdot \mu^{-1}$.

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АНАЛІТИЧНА РОЗРОБКА ТА ВАЛІДАЦІЯ ПРОСТИХ ПРОЦЕДУР ВИЗНАЧЕННЯ МЕЗЛОЦИЛІНУ ДВОМА АЛЬТЕРНАТИВНІМИ МЕТОДАМИ

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У статті розглядається пошук нових аналітичних реакцій та з'ясування оптимальних умов їх перебігу, які можуть бути покладені в основу кількісних аналітичних визначень пеніцилінів. Розроблено дві уніфіковані методики та можливість кількісного визначення методами кінетико-спектрофотометрії та окисно-відновного титрування мезлоциліну в чистій субстанції та препараті з використанням калій кароату. Запропоновано схему хімічного перетворення мезлоциліну. Досліджено кінетику спряжених реакцій S-окиснення та пергідролізу мезлоциліну у лужному середовищі за збільшенням світлопоглинання продукту утворення при 290 нм. Поява нової хвилі дає можливість розробки нової методики кількісного визначення мезлоциліну. Швидкість реакції контролювали спектрально та відображали в реальному часі. Для обробки кінетичних даних використовувався диференціальний варіант методу дотичної.

Ключові слова: антибіотик, мезлоцилін, метод кінетико-спектрофотометрії, метод окисно-відновного титрування, пергідроліз, калій кароат.

ANALYTICAL DEVELOPMENT AND VALIDATION OF SIMPLE PROCEDURES FOR THE DETERMINATION OF MEZLOCILLIN BY TWO ALTERNATIVE METHODS

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This article addresses the search for new analytical reactions and the determination of optimal conditions for their implementation, which can serve as the basis for quantitative analysis of penicillins. Two unified procedures were developed, and the possibility of quantitative determination of mezlocillin in a pure substance and pharmaceutical formulation by kinetic spectrophotometry and redox titration using potassium caroate was demonstrated. A scheme of the chemical transformation of mezlocillin in the reaction with potassium caroate is proposed. The kinetics of the coupled reactions of S-oxidation and perhydrolysis of mezlocillin with potassium caroate in an alkaline medium were studied by monitoring the increase in absorbance of the formed product at 290 nm. The appearance of a new absorption band enabled the development of a new procedure for the quantitative determination of mezlocillin. The reaction rate was monitored spectrally and displayed in real time. A differential variation of the tangent method was used to process the kinetic data.

Keywords: antibiotic; mezlocillin; kinetic spectrophotometry; redox titration; perhydrolysis; potassium caroate.

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