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QUANTITATIVE DETERMINATION OF AMOXICILLIN BY SPECTROPHOTOMETRIC METHOD USING POTASSIUM CAROATE

Despite the fact that many different physicochemical methods are used in the practice of analysis, the task of improving known and developing new methods for quantitative determination of penicillins remains relevant in the future. Existing pharmacopoeial methods for determining drugs of this series are quite complex, time-consuming, and require the use of sophisticated, expensive equipment. The disadvantage of the known, fairly simple methods for spectrophotometric determination of penicillins, which are reduced to determining the final products of their hydrolytic cleavage, is the need for prolonged heating.

Aim: *development of new improved unified method for the quantitative determination of Amoxicillin (Amox).*

Materials and methods. *The object of the study was Amox sodium powder in vials for the preparation of an injection solution («Amoxicillin Sodium Salt», 1000 mg). Peroxomonosulfate acid as triple potassium salt $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$ (Oxone®) of "extra pure" qualification was used as oxidant. Using the method of kinetic spectrophotometric, procedure for the quantitative determination of Amox in the substance and the drug product have been developed using potassium caroate as an analytical reagent ($KHSO_5$).*

Results and discussion. *A unified methodology was developed and the possibility of qualitative determination of Amox in pure substance and drug using potassium caroate was investigated by kinetic-spectrophotometry. The scheme of the chemical transformation of Amox with the reaction of potassium caroate has been proposed. The kinetic of the conjugated reactions of S-oxidation and perhydrolysis of Amox with potassium caroate in alkaline medium is studied by the increase of forming product light absorbance at 296 nm. The appearance of a new wave gives the possibility of developing a new procedure for the quantitative determination of Amox. The reaction rate was monitored spectrally and display in real time. A differential variation of the tangent method was used to process the kinetic data.*

Conclusions. *Using the kinetic-spectrophotometry method, a method for the quantitative determination of amoxicillin in the substance was developed and the drug product have been developed using potassium caroate as an analytical reagent ($KHSO_5$). The developed method of quantitative determination of Amox 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.*

Key words: *oxidation, kinetic-spectrophotometry, validation, potassium caroate, amoxicillin.*

Світлана Карпова, Ольга Антоненко. КІЛЬКІСНЕ ВИЗНАЧЕННЯ АМОКСИЦИЛІНУ СПЕКТРОФОТОМЕТРИЧНИМ МЕТОДОМ ЗА ДОПОМОГОЮ КАЛІЙ КАРОАТУ

Незважаючи на те, що в практиці аналізу використовують багато різноманітних фізико-хімічних методів, завдання вдосконалення відомих та опрацювання нових методик кількісного визначення пеніцилінів залишається актуальним і надалі. Існуючі фармакопейні методики визначення препаратів цього ряду достатньо складні, довготривалі та вимагають використання складної висококоштовної апаратури. Недоліком відомих достатньо простих у виконанні методик спектрофотометричного визначення пеніцилінів, які зводяться до визначення кінцевих продуктів їх гідролітичного розщеплення, є необхідність тривалого нагрівання.

Мета роботи: *розробка нової вдосконаленої уніфікованої методики кількісного визначення амоксициліну.*

Матеріали та методи. *Об'єктом дослідження був порошок натрій амоксициліну у флаконах для приготування розчину для ін'єкцій («Амоксициліну натрієва сіль», 1000 мг). Як окисник використовували пероксомоносульфатну кислоту у вигляді потрійної калієвої солі $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$ кваліфікації "extra pure" (Oxone®). Використовували метод кінетико-спектрофотометрії для розроблення методики кількісного визначення амоксициліну в субстанції та лікарському засобі з використанням калій кароату як аналітичного реагенту ($KHSO_5$).*

Результати та їх обговорення. *Розроблено уніфіковану методику та досліджено можливість кількісного визначення методом кінетико-спектрофотометрії амоксициліну в чистій речовині субстанції та препараті з використанням калій кароату. Запропоновано схему хімічного перетворення амоксициліну з реакцією кароату калію. Кінетику спряжених реакцій S-окиснення та пергідролізу амоксициліну з калій кароатом у лужному середовищі досліджують за збільшенням поглинання світла продуктом, що утворюється, при 296 нм. Поява нової хвилі дає можливість розробити нову процедуру кількісного визначення амоксициліну. Швидкість реакції контролювали спектрально та відображали в режимі реального часу. Для обробки кінетичних даних використовували диференціальну варіацію тангенційного методу.*

Висновки. *Використовуючи метод кінетико-спектрофотометрії, була розроблена методика кількісного визначення амоксициліну в субстанції та лікарському засобі з використанням калій кароату як аналітичного реагенту ($KHSO_5$). Розроблена методика кількісного визначення амоксициліну може бути використана для розробки аналітичної нормативної документації на лікарські засоби, а також у практиці державних лабораторій з контролю якості лікарських засобів та центральних заводських лабораторій фармацевтичних підприємств.*

Ключові слова: *окиснення, кінетико-спектрофотометрія, валідація, калій кароат, амоксицилін.*

Introduction. Amoxicillin is a widely utilized beta-lactam antimicrobial drug approved by the U.S. Food and Drug Administration (FDA) for use in the primary care setting. Amox is an aminopenicillin created by adding an extra amino group to penicillin to battle antibiotic resistance. Amox is effective against a wide range of gram-positive bacteria, offering additional coverage against some gram-negative organisms compared to penicillin. This activity delves into the indications, mechanism of action, administration, contraindications, and adverse event profiles associated with Amox. This activity equips clinicians with a comprehensive understanding of amoxicillin to optimally enhance their ability to manage infectious diseases in patients [8].

Amox shown in Fig. 1 is chemically known as by IUPAC Name (2S,5R,6R)-6-[[(2R)-2-amino-2-(4-hydroxyphenyl) acetyl] amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicycloheptane-2-carboxylate.

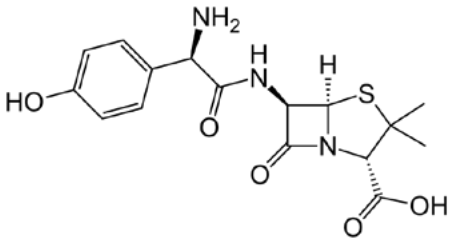

Fig. 1. Chemical structures of Amoxicillin

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 have to be controlled. Therefore, 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 to determine penicillin summary in semi-synthetic penicillin by neutralization method after preparation hydrolysis by excess of sodium hydroxide titrated solution at heating [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.

Well, described in scientific articles methods of potentiometry titration, amperometry, high-performance liquid chromatography (HPLC), voltammetry, polarographic analysis, micelle electrokinetic capillary, spectrophotometry, chemiluminescense, iodometry and others [1–5, 7, 9–11] for the quantitative determination of penicillin drugs.

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 Amox developed by us have a number of advantages over the already known ones: they allow determining Amox 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. Also, 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 Amox using potassium caroate [12–15].

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

For the research, Amoxicillin sodium salt of pharmacopoeial purity, a dry sterile powder in vials (1000 mg) for inejction «Amoxicillin Sodium Salt», CAS Number 34642-77-8, Catalog Number A-551-1, Jiangxi Bolai Pharmacy Co., Ltd, China. 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. 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

$$HSO_{5^-} + 2H^+ + 2\bar{e} \rightarrow HSO_{4^-} + H_2O$$

is 1.81 V.

Working solution of potassium caroate, 2×10^{-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 °C. The solution concentration was controlled by iodometric titration.

As a standard sample of Amoxicillin sodium salt, we used the substance of Amox of pharmacopoeial purity with the content of the main substance of 98.5%.

Standard sample solution of Amox, 1×10^{-2} mol·L^{-1}. 0.3874 g of Amoxicillin sodium was diluted in double-distilled water in a 100 mL flask at 20 °C.

Working solutions of Amox. Seven aqueous solutions of the following concentrations (%): 60; 80; 100; 110; 120; 130; 150 were prepared in 100 mL volumetric flasks; the corresponding portions of 0.2324; 0.3099; 0.3874; 0.4261; 0.4649; 0.5036; 0.5811 of the Amox substance were weighed (g).

Sodium hydroxide solution, 5.5×10^{-2} mol·L^{-1}. The sodium hydroxide solution was prepared according to Hillebrant by diluting the saturated solution with freshly distilled water.

Spectrophotometry. The spectra of solutions of Amox 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).

Kinetic Spectrophotometric Method. Close 50 mg (accurate weight) of the powder of the Amox 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, 4.0 mL of a 2×10^{-2} mol·L^{-1} $KHSO_5$ solution and 4.0 mL of NaOH with the concentration of 5.5×10^{-2} mol·L^{-1} was added. The resulting solution was exposed to photometric measurements for 10 min in a 1 cm quartz cuvette at 296 nm using distilled water as a compensation solution.

Results and Discussion. The effect of Caro's acid. 1 mL of 1×10^{-2} mol·L-1 solution of Amox was pipetted into 100 mL volumetric flasks containing 1 mL, 2 mL, 3 mL, 4 mL, 5 mL and 7 mL of 2×10^{-2} mol·L^{-1} $KHSO_5$ solution and 1 mL of 5.5×10^{-2} mol·L^{-1} NaOH solution. The content of the mixture of each flask was mixed well, and the increase in absorbance at 296 nm was recorded for 30 min against the reagent blank as a function of time. It showed the dependence of absorption at 296 nm of Amox 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 4.0 mL of 2×10^{-2} M Caro's acid was used. Thus, 4 mL of 2×10^{-2} M Caro's acid was chosen as the optimal value.

The effect of the Sodium Hydroxide concentration. 1 mL of 1×10^{-2} mol·L-1 solution of Amox was pipetted into 100 mL volumetric flasks containing 0,5 mL, 1mL, 2 mL, 3 mL, 4 mL, 5 mL and 7 mL of 5.5×10^{-2} mol·L^{-1} NaOH solution of 2×10^{-2} mol·L^{-1} $KHSO_5$ solution. The content of the mixture of each flask was mixed well, and the increase in absorbance at 296 nm was recorded for 30 min against the reagent blank as a function of time. It showed the dependence of the absorption at 296 nm of Amox 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 4 mL of 5.5×10^{-2} mol·L^{-1} NaOH was used. Thus, 4 mL of 5.5×10^{-2} mol·L^{-1} NaOH was chosen as the optimal value.

The 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 Amox 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. 2). 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.

Plotting a calibration graph. Using a microburette, 0.50; 2.50; 3.00; 4.00; 5.00; 6.00 mL samples of the standard Amox solution were added to 50 mL volumetric flasks followed by 4 mL of $2\cdot10^{-2}$ mol·L^{-1} $KHSO_5$ solution put to each flask, and the content was shaken

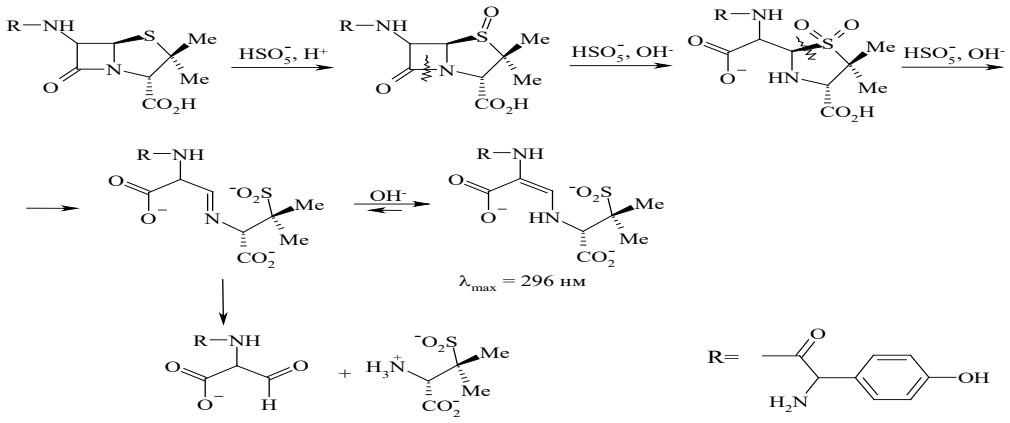

Fig. 2. The scheme of coupled reactions of peroxyacid oxidation and perhydrolysis of sulfon Amox with the formation of a substituted derivative of N-acryl-β-penicylamine sulfate

thoroughly. 4.0 mL of 5.5×10^{-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 296 nm against distilled water (compensation solution) for 10 minutes every minute at 20°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 tgα on the concentration of Amox (c, μg·mL^{-1}) was constructed.

Fig. 3 shows a calibration graph for determining Amox, according to which, the dependence of concentration on tgα is linear in the range of 5 to 50 μg·mL^{-1}. This makes it possible to determine the quantitative content of Amox in the given concentration range by the standard method.

The content of $C_{16}H_{19}N_3O_5S$, in mg in one vial, (X_{Amox}) was calculated by the formula:

$$X_{\text{Amox}} = \frac{a_{st} \cdot \text{tg}\alpha \cdot \bar{a} \cdot w}{\text{a} \cdot \text{tg}\alpha_{st}},$$

where: a_{st} – is the mass of a standard sample of Amox sodium salt, mg;

tga_{st} – is the tangent of the angle of the slope of the kinetic curve in the study with the standard solution of Amox sodium salt, min^{-1};

w – is the content of $C_{16}H_{19}N_3O_5S$ Amox sodium salt in the standard sample of Amox, in mass fractions;

a – is the weighed portion of the powder of Amox sodium salt studied, mg;

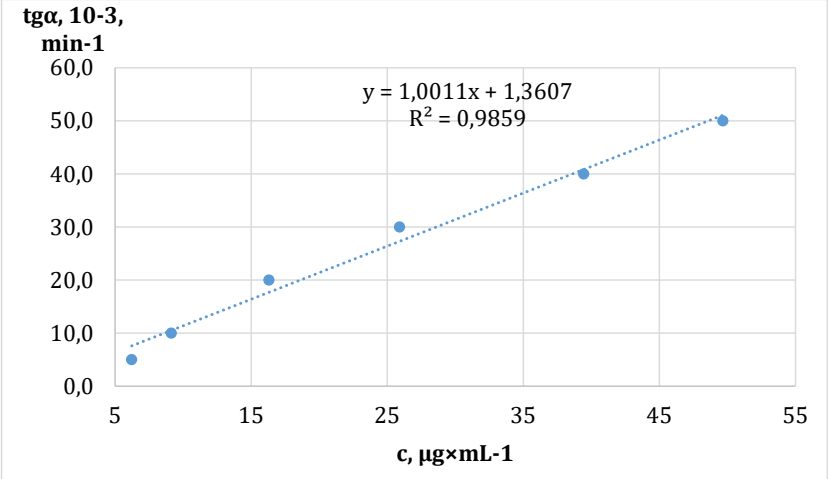

Fig. 3. The calibration graph for the quantitative determination of Amox, c(NaOH) = 5.5×10^{-2} mol·L^{-1}; c($KHSO_5$) = 2.0×10^{-2} mol·L^{-1}

Table 1

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

Amoxicillin taken, mg	Found		Results of processing statistical data
	mg	%	
935.0[a]	945.2	94.5	$\bar{x}$ = 942.3 (94.2%) S = ± 7.93549 S_x = ± 2.99933 $\Delta\bar{x}$ = ± 7.34836 RSD = ± 0.84% ε = ± 0.78% δ[b] = + 0.78%
	952.4	95.2	
	949.3	94.9	
	937.7	93.8	
	928.3	92.8	
	941.2	94.1	
	942.1	94.2	

Note: [a] The Amox content indicated in the quality certificate (μ); [b] $\delta = (\bar{x} - \mu) \times 100\% \times \mu^{-1}$.

$\bar{a}$ – is the average weight of the drug in the vial, mg;

tg$a$ – is the tangent of the angle of the slope of the kinetic curve in the study with the test solution of A_{mox} sodium salt, min^{-1}.

The results of the analysis of the A_{mox} drug by kinetic spectrophotometric method are shown in Table 1. The relative standard deviation did not exceed 0.84% (δ = + 0.78%).

Conclusions. The possibility of analytical determination of Amoxicillin 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 Amoxicillin, 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 kinetic-spectrophotometry method, a method for the quantitative determination of amoxicillin in the substance was developed and the drug product have been developed using potassium caroate as an analytical reagent ($KHSO_5$). The developed method of quantitative determination of Amoxicillin 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.

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