IJPSR (2018), Volume 9, Issue 8 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 15 November, 2017; received in revised form, 11 June, 2018; accepted, 06 July, 2018; published 01 August, 2018

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC AREA UNDER CURVE METHOD QUANTITATIVE ESTIMATION OF PIPERACILLIN

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Keywords:

Spectrophotometry, Piperacillin, Perhydrolysis, Validation, Potassium hydrogen peroxomonosulfate

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ABSTRACT: The kinetic of conjugated reactions of S-oxidation and perhydrolysis of Piperacillin (Pip) with potassium hydrogen peroxo-monosulfate in alkaline medium is studied by the increase of forming product light absorbance. A scheme of peroxoacid oxidation and perhydrolysis conjugated reactions of Piperacillin by means of hydrogen peroxomonosulfate is proposed. The procedure of the quantitative analysis of Piperacillin in the combined with Tazobactam pharmaceutical preparation powder of Zopercin® (Piperacillin 4.0 g and Tazobactam 0.5 g) by spectrophotometric - kinetic method is elaborated using triple potassium Caro's salt solution as a reagent. Peroxomonosulfate acid as triple potassium salt $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$ (Oxone®) of "extra pure" qualification was used as oxidant. 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 min (observation time). The method on initial rates (tangent method) was used to collect kinetic data (usually at 280 nm) by following the appearance product of perhydrolysis reaction of Piperacillin. A solution of sodium hydroxide was thermostated in the cell compartment, and then mixtures of solutions of Piperacillin / Tazobactam with solutions Caro's acid (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 ± 1-3%. The results were obtained by the recommended procedure for seven replicate titrations of mixtures containing the three species at various concentrations. RSD = 1.4%. The obtained results have good agreement with the Parmacopoeia one δ = 1.0%.

INTRODUCTION: Piperacillin/Tazobactam is a combination antibiotic containing the extended-spectrum penicillin antibiotic Piperacillin and the *β*-lactamase inhibitor Tazobactam (Taz) and is used to reduce the development of drug - resistant bacteria.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(8).3556-60

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(8).3556-60

[2S- [2a, 5a, 6b(S)]]-6-[[[(4-ethyl-2, 3-dioxo -1-piperazinyl) carbonyl] amino] phenil-acetyl] amino -3, 3dimthyl- 7-oxo-4- thia-1-azabicyclo- [3.2.0] heptanes-carboxylicacid (Pip) belongs to the ureidopenicillin class and it is used for the treatment of serious infections caused by susceptible strains of microorganisms. (2S,3S,5R)-3-methyl- 7-oxo-3-(1H-1, 2, 3-triazolylmethyl)-4-thia-1- azabicyclo- [3.2.0] heptanes-2-carboxylic acid-4, 4-dioxide (Taz) is used in combination with beta-lactamase antibiotic as antibacterial. Preparations of penicillin family are derivates of 6-aminopenicillanic acid (6-APA), a condensed

system of thiazolidine and *β*-lactam tetramine cycles that differ by the radical R connected with 6-APA amino group [1]. 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 [2].

The extensive literature survey reveals varies methods of quantitative determination of penicillin family preparations, such as HPLC, spectro-photometry, iodometry, extraction photometry, different variants of voltametry, polarography and kinetic analysis are proposed. The spectro-photometric methods that are based on the application of phenol Folin-Ciocalteu reagent, reactions with Mn(II), Co(II) and Ni(II) salts and etc. are also known. These methods give the possibility to determine penicillin in medical preparations in presence of different excipients [3-15].

Thus, the improvement of the known and development of new methods of quantitative determination of penicillin is rather important. The existing Pharmacopoeial methods of Penicillin preparations determination are quite complex, long-lasting and require the application complex and expensive devices. The disadvantage of the known simple enough in performance methods of spectrophotometric determination of penicillin, which are based on the determination of the final products of their hydrolytic cleavage, is the requirement of prolonged heating [16].

The developed method of Piperacillin (Pip) kinetic determination has several advantages: makes it possible to identify the preparation in much smaller quantities than the Parmacopoeial iodometric method, it is applicable to the same range of concentrations, as in photometric determination of hydrolysis products, but it doesn't require prolonged heating of the reaction mixture, it is simpler and faster than the method of chromatographic analysis. It is based on the preliminary oxidation of Piperacillin (Pip) with potassium hydrogen peroxomonosulfate excess to the corresponding S-oxide, followed by determination of the hydrolytic conversion of it's product in an alkaline medium by the kinetic spectrophotometric method [Initial rate (tangent) method].

MATERIALS AND METHODS: All the materials were of analytical reagent grade, and the solutions were prepared with double-distilled water. Piperacillin sodium salt substance (CAS Number 59703-84-3) sodium (2*S*,5*R*,6*R*)-6-{[(2*R*)-2-[(4-ethyl-2, 3-dioxo- piperazine -1- carbonyl) amino]-2- phenyl-acetyl]amino}-3, 3-dimethyl-7-oxo-4- thia-1- azabicyclo[3.2.0] heptane-2-carbo xylate ($C_{23}H_{26}N_5NaO_7S$).

Zopercin® - powder in vials for Piperacillin injection solution in the combined form with Tazobactam (Piperacillin 4.0 g and Tazobactam 0.5 g). Manufacturer Orchid Healthcare (Office Orchid Chemicals and Pharmaceuticals Limited, India), series no. UA/5033/01/01 **Fig. 1**.

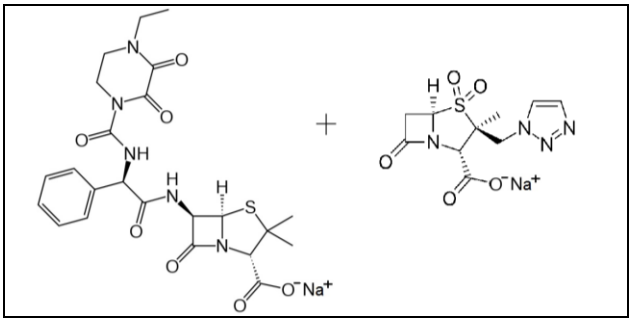


FIG. 1: CHEMICAL FORMULAS OF ZOPERCIN PREPARATION CONTENT

Peroxomonosulfate acid 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, has good solubility and stability in water. It was proposed for cefadroxil kinetic spectrophotometric determination as an analytical reagent. Standard electrode potential for redox semireaction is 1.8 V [17].

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

Standard Solution of Piperacillin Sodium (600 µg mL^{-1}): 600 mg of piperacillin sodium was transferred in 100 mL volumetric flask and diluted to the mark with double distilled water at 20 ºC.

Standard Solution of Peroxomonosulfate ($2 \cdot 10^{-2}$ mol L^{-1}): 0.615 mg of oxone was transferred in

100mL volumetric flask and diluted to the mark with double distilled water at 20 ºC. The solution of peroxomonosulfate 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^{-1} solution of sodium hydroxide without carbonates was used to creat 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 ($tg\alpha_{pip}$, min^{-1}). Kinetic measurements were made by using a Cary 15 recording spectro-photometer which a constant-temperature bath connected to the cell holder. Measurements of the pH were made on potentiometer (Gomel, Belarus, I-160 Model).

Initial Rate Method: The method on initial rates (tangent method) was used to collect kinetic data (usually at 280 nm) by following the appearance product of perhydrolysis reaction of Piperacillin. A solution of sodium hydroxide was thermostated in the cell compartment, and then mixtures of solutions of Piperacillin/Tazobactam with solutions Caro's acid (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 ± 1-3%.

Aliquots of 0.50-10.00 mol L^{-1} of the studied Pip test solutions were pipetted into a series of 50 mL volumetric flask containing 5 mL of $2 \cdot 10^{-2}$ mol L^{-1} $KHSO_5$ solution and mixed well. 5 mL of 0.06 mol L^{-1} 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 280 nm was recorded as a function of time for 10 minutes against reagent blank. It shows the dependence of Pip alkaline solutions absorption against time at 280 nm. They have linear dependence during 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 Pip (C, µg mL^{-1}). For simplicity, we use the following terminology. The peroxomonosulfate (both HSO_5^- and SO_5^{2-}) is termed caroate system, the perhydrolysing mixture of peroxomonosulfate with NaOH solution will be ermed the perhydrolysis system SO_5^{2-}. Piperacillin and Tazobactam will be symbolized Pip and Taz, respectively. $SO_5^{2-} + HO^-$ was considered to be the active perhydrolysant in these reactions, and the data are consistent with this interpretation. Also a preliminary kinetic study by iodometric titration method was conducted on the S-oxidation of Pip by the peroxomonosulfate system; the reaction is

$$-S- + HSO_5^- \rightarrow -S(O)- + H^+ + SO_4^{2-}$$

A mechanism involving the intermediate S-oxide Pip (formed in a rapid step), and its conjugate with perhydrolysis system was postulated. Product per hydrolysis reaction of Pip (λ_{max} 280 nm).

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 Piperacillin under study with potassium hydrogen peroxomonosulphate, and therefore with alkali solution. Maximum activity of potassium hydrogen peroxomonosulfate in the reaction was achieved at concentrations $2 \cdot 10^{-3}$ $molL^{-1}$. The electronic spectra of interaction product of Piperacillin with reagents depending on the time are shown on **Fig. 2**.

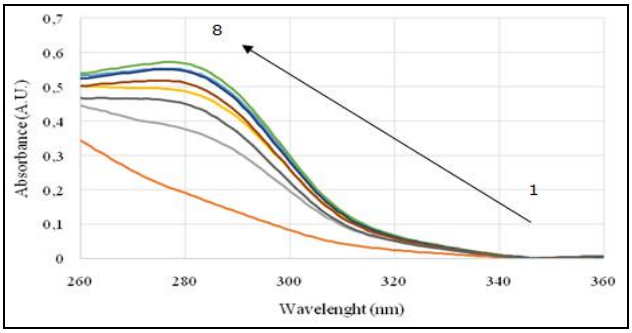
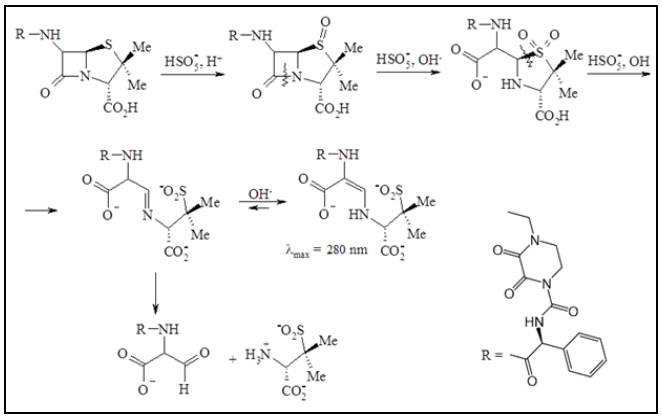


FIG. 2: UV ABSORPTION SPECTRA OF SYSTEM OF $1 \cdot 10^{-4}$ mol/l PIPERACILLIN WITH $2 \cdot 10^{-3}$ mol/l POTASSIUM HYDROGEN PEROXOMONOSULFATE IN 0.01 mol/l NaOH AS FUNCTION OF TIME (min): 1 - 3; 2 - 7; 3 -11; 4 - 15; 5 - 19; 6 - 23; 7 - 27; 8 - 31

The theoretical scheme of transformation of the reaction product is given on **Scheme 1**:



SCHEME 1: THE SCHEME OF PEROXO ACID OXIDATION AND PERHYDROLYSIS CONJUGATED REACTIONS OF PIPERACILLIN

The calibration plot for kinetic determination of Pip in optimum conditions is given on the **Fig. 3**. The tgα linear concentration dependence was observed within the Pip content in solution 1-50 µg mL^{-1}. This was a precondition for the possibility of using the kinetic method in the analysis.

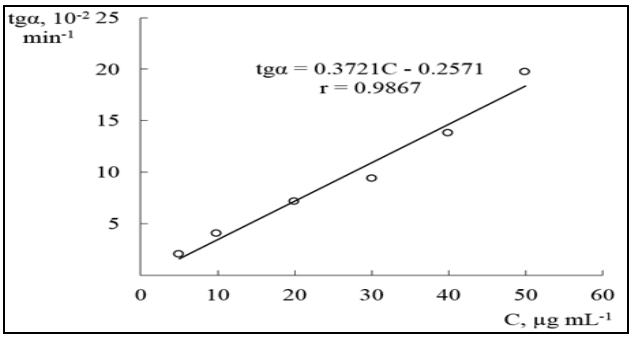


FIG. 3: THE CALIBRATION PLOT FOR KINETIC DETERMINATION OF PIPERACILLIN USING POTASSIUM HYDROGEN PEROXOMONOSULFATE

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 Piperacillin could be determined successively with good accuracy and reproducibility.

TABLE 1: DETERMINATION OF PIPERACILLIN BY KINETIC METHOD WITH USE $KHSO_5$ AS OXIDIZING AGENT

Taken mg	Determined by kinetic method,* $\bar{X} \pm \Delta\bar{X}$	RSD (%)	$\delta = \frac{x - a}{a} \times 100$	Recovery kinetic method (%)
1.428	1.43 ± 0.037	2.79	0.14	100.14
2.735	2.74 ± 0.041	1.61	0.18	100.18
5.413	5.42 ± 0.046	0.92	0.13	100.13

* Average of seven determinations (P = 0.95).

Procedure of Piperacillin Assay in Flacons: Piperacillin sodium (ca 600 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. The resulting solution was measured photometrically in a quartz cuvette at 280 nm against distilled water (compensation solution) during first 15 min every minute and the absorbance kinetic curves against time was constructed. The slop of the linear section of the kinetic curve, *tgα* was determined. The content of $C_{23}H_{26}N_5NaO_7S$, in g, in one flacon (X_{Pip}) was calculated using the euqation:

$$X_{Pip} = \frac{a_{st} \cdot tg\alpha \cdot \bar{a}}{a \cdot tg\alpha_{st}}$$

Where, a_{st} – the sample weight of the work standard of Piperacillin, g; tgα_{st} – the slope ratio of the kinetic curve in the experiment with the work standard of piperacillin, min.$^{-1}$; a – the sample weight of the studied powder of piperacillin, g; $\bar{a}$ – the average weight of the flacon, g; tgα – the slope ratio of the kinetic curve in the experiment with the Piperacillin solution, min.$^{-1}$.

The results of the Pip quantitative determination are given in the **Table 2**. The proposed method has good accuracy, RSD = 1.4 %.

TABLE 2: RESULTS OF PIPERACILLIN QUANTITATIVE DETERMINATION BY MEANS OF POTASSIUM HYDROGEN PEROXOMONOSULFATE (P=0.95, n=7)

Nominal piperacillin mass, g	Actual g	Actual g	Actual
Zopercin® Orchid Healthcare (India)			
4.001*	4.1028	102.54	$\bar{x}$ = 4.0411(101.00%)
Series no.	3.9977	99.92	S = 0.0568
UA/5033/01/01	4.0945	102.34	S_x = 0.0215
	4.0321	100.78	$\Delta\bar{x}$ = 0.0527
	3.9589	98.95	RSD = 1.4 %
	4.0075	100.16	δ = 1.0 %
	4.0945	102.34	

Content of Pip in preparation was controlled by the independent method of iodometric titration.

CONCLUSION: The reaction kinetics of the peroxyacidic oxidation and perhydrolysis of Piperacillin with potassium peroxomonosulfate in the alkaline medium is 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 Piperacillin in the Zopercin® preparation based on the results of the kinetic-spectrophotometric method with potassium peroxomonosulfate as reagent was shown. RSD = 1.4 %, δ = 1.0%.

ACKNOWLEDGEMENT: I acknowledge my parents, husband, seniors and my colleagues for their support and motivation.

CONFLICT OF INTEREST: The authors do not have any conflict of interest.

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How to cite this article:
Karpova SP, Blazheyevskiy MY and Mozgova OO: Development and validation of UV spectrophotometric area under curve method quantitative estimation of piperacillin. Int J Pharm Sci & Res 2018; 9(8): 3556-60. doi: 10.13040/IJPSR.0975-8232.9(8).3556-60.