Validation of Amoxicillin iodometric procedure in quantitative analysis of pure substance and medical preparation

Yu. Yu. Serdiukova, O. V. Kolisnyk, T. O. Tomarosvka, S. M. Poluian, Z. V. Shovkova, O. H. Pohosian

National University of Pharmacy, Kharkiv, Ukraine

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

The aim of the work is to validate a simple and rapid iodometric procedure for the quantitative determination of amoxicillin in pure substance and medicinal preparation using potassium caroate as analytical reagent.

Materials and methods. The procedure involves the use of potassium caroate (KHSO₅) as an oxidant. The assay is based on the quantitative penicillin oxidation by KHSO₅ to the corresponding S-oxide. The interaction between amoxicillin and analytical reagent is stoichiometric. Indirect iodometric method is used for the quantitative determination of amoxicillin main substance. The validation procedure was performed according to the State Pharmacopeia of Ukraine.

Results. The precision, accuracy, limit of detection (LOD), and limit of quantitation (LOQ) are accepted over the concentration range of 80–120 % with a correlation coefficient of 0.999. LOD and LOQ were found to be 4.91 % and 14.73 % for amoxicillin pure substance respectively. The precision calculated as the relative standard deviation (RSD) was less than 0.8 % and accuracy (δ, relative error) was better than 0.4 %. The proposed method was validated statistically and through recovery studies. For Amoxicillin medical preparation RSD ≥ 1.93 % and δ ≥ 1.62 %.

Conclusions. The obtained data showed acceptable agreement with the certificate results, so the proposed procedure can be used for the assay of amoxicillin in medicinal preparation.

Key words: analysis, validation, amoxicillin, potassium caroate.
Penicillin is widely used in nowadays treatment of various diseases. It belongs to a β-lactam group of antibiotics. The modern literature reveals the following procedures proposed for quantitative analysis of Amoxicillin assay in bulk and medicinal preparation. There are chromatography procedures [1, 2]. The techniques are accurate and precise but require long-lasting preparation and expensive reagents. The titrimetric procedures [3] are developed for some penicillins. A blank determination is required. Voltammetric [4, 5], spectrophotometric [6, 7], and chemiluminescence [8, 9] procedures are also described in modern research results. They are sensitive enough, show precise and repeatable data. The proposed methods require application of additional equipment which is not suitable for small laboratories. HPLC is a pharmacopeial method recommended for the penicillin assay [10–12].

The reverse iodometric titration is used for the quantitative determination of penicillins. The titer of the procedure depends on temperature and should be corrected each time. The experiment performance is approximately 40 min [13].

So, the titration procedures can be applicable for Amoxicillin assay because of their simplicity, economic profit, and duration.

One of the advantages of the developed procedures is the usage of one oxidation reagent for the determination of different β-lactams. This reagent is nonpoisonous, low-cost, stable while stored storage for a long time. Potassium caroate as an analytical reagent meets all of the requirements. That is why validation of Amoxicillin pure substance and medical preparation reverse iodometric titration quantitative determination by means of potassium caroate is of great interest.

Aim
The aim of this work is the investigation a reaction of Amoxicillin with potassium caroate and validation of the developed iodometric procedure for pure substance and medical preparation.

Materials and methods

All used reagents were of a chemical purity.

Potassium caroate (KHSO$_4$) solution was used as an analytical reagent. Caro acid is commercially available under the trade name “Oxon”. The 0.02 mol L$^{-1}$ solution of potassium caroate is stable enough (the oxidation activity remains constant during 30 days). It is nontoxic and not expensive.

1. Preparation of 0.02 mol L$^{-1}$ potassium caroate solution. 0.615 g (the precise weight) of KHSO$_4$, are dissolved in 100 mL of distilled water at 293 K. The reverse iodometric titration is used for blank determination of potassium caroate content.

2. Preparation of 0.02 mol L$^{-1}$ sodium thiosulphate solution. The standard titer fixanal was used for preparation of the 0.1 mol L$^{-1}$ sodium thiosulphate solution at 293 K.

3. Preparation of 5% potassium iodide solution. 5.0 g of potassium iodide was dissolved in 100 mL volumetric flask in distilled water.

4. Preparation of 0.1 mol L$^{-1}$ sulfuric acid solution. The standard titer fixanal was used for the preparation of the solution.

The microburette with the ±0.01 mL accuracy was used for iodimetric investigation.

Amoxicillin pure substance was used as received. The content of the main substance was determined independently by the method of HPLC and given in the quality certificate, together with the moisture. Amoxicillin medical preparation was used in the form of capsules (0.5 g, produced by TEVA, France). The quality certificate data were obtained by the HPLC method (the content of Amoxicillin is 0.491 g).

The procedure of Amoxicillin assay using HPLC method. Liquid chromatography (2.2.29) as described in the test for related substances with the following modifications. Mobile phase...
Initial composition of the mixture of mobile phases A and B, adjusted where applicable. Injection Test solution (a) and reference solution (a). System suitability Reference solution (a): – repeatability: maximum relative standard deviation of 1.0 percent after 6 injections.

Calculate the percentage content of $C_{16}H_{19}N_3O_5S$ from the declared content of amoxicillin trihydrate CRS [10–12].

**Investigation of S-oxidation reaction of Amoxicillin with potassium caroate.** 10.0 mL of 0.02 mol L$^{-1}$ of potassium caroate solution and 10.0 mL of 0.01 mol L$^{-1}$ penicillin solution were pipetted into 100 mL volumetric flask and brought to the mark with distilled water. After the addition of Amoxicillin, the stopwatch was switched on. The volume was mixed. After certain periods of time 10 mL of the obtained mixture were taken for titration into flask containing 1 mL of sulfuric acid solution and 1 mL of potassium iodide solution. The 0.02 mol L$^{-1}$ sodium thiosulphate solution was used for titration of isolated iodine in the presence of starch.

The procedure of Amoxicillin pure substance determination using potassium caroate. 0.35 g of penicillin were dissolved by heating in 10 mL of DMFA 100.00 mL volumetric flask. After the complete dissolution, the volume was brought to the mark with distilled water. Further like in the S-oxidation reaction of Amoxicillin.

The blank investigation was performed in the same conditions paralleled (without Amoxicillin with the same amount of KH$\text{SO}_4$ 0.02 mol L$^{-1}$ solution).

The procedure of Amoxicillin medical preparation determination using potassium caroate. The content of the capsule was dissolved in 10 mL of DMFA by heating in 100.00 mL volumetric flask and brought to the mark with distilled water. Further like in the Amoxicillin pure substance.

**Method Validation.** The method was validated according to the State Pharmacopeia of Ukraine article “Validation of analytical methods” [14]. The following criteria were analyzed: specificity, accuracy, precision, linearity, range, detection limit, quantitation limit. Microsoft® Excel 2010 was used for the calculation of regression parameters.

**Results**

The procedure is based on the reaction of amoxicillin S-oxidation using potassium caroate in acidic medium. The scheme of the hypothetic reaction is assumed from literature survey and our investigations and is shown in the **Fig. 1**.

The excess of potassium caroate was determined by reverse iodometric titration.

Each one mL of sodium thiosulphate 0.01 mol L$^{-1}$ solution is equivalent to 0.001827 g of amoxicillin preparation.

The recommended range of titration procedure is the interval from 80 % to 120 %. The straight linear dependence was investigated for five working solutions with the concentrations 80 %; 90 %; 100 %; 110 % and 120 %. The titration was repeated three times for every concentration. The results obtained were analyzed by the least square method for the straight linear dependence: $Y = b \cdot X + a$. They are shown in **Table 1**. The linearity was studied in the normalized coordinates and is proposed in **Fig. 2**.

The precision and ruggedness of the proposed procedure were studied by measuring five different concentrations with three times repetition. Accuracy and convergence were studied using the same working solutions. The obtained results were analyzed statistically. The received data were compared to the SPhu criterion. The results are shown in **Table 2**.

The results of Amoxicillin capsules quantitative determination using potassium caroate as an analytical reagent by the iodometric procedure are given in **Table 3**. The investigation was performed for 3 different concentrations and robustness was studied for two days.

**Discussion**

The S-oxidation reaction between Amoxicillin and potassium caroate is quantitative and stoichiometric: 1 mol of potassium caroate goes per 1 mol of Amoxicillin. Amoxicillin S-oxide is the product of redox interaction. The duration of interaction is not more than 1 min [3].
Table 1. Characteristic parameters of the linearity of amoxicillin assay using potassium caroate (Y = b·X + a)

| Parameter | Value | Standard deviation (SD) | Statistical uncertainty criterion (≤ 1.21·SD) | Practical acceptability criterion | Conclusion |
|-----------|-------|--------------------------|---------------------------------------------|----------------------------------|------------|
| a         | 1.4735| S_a = 1.465               | | | corresponds |
| b         | 0.9850| S_b = 0.015               | | | corresponds |
| S_rest    | 0.465 |                         | ≤0.48                                      | ≥0.99969                        | corresponds |
| R         | 0.99969|                         |                                             |                                |            |
| LD        | 4.91 %|                          |                                             |                                |            |
| LOQ       | 14.73 %|                          |                                             |                                |            |

Table 2. The results of validation parameters analysis for Amoxicilline iodometric determination using potassium caroate

| No. of working solution | Nominal x, % | Titrant volume (V_f – V_i), mL | Actual y_i (%) | Actual in compare with nominal, % Z_i = 100 (Y_i / X) |
|-------------------------|--------------|---------------------------------|----------------|------------------------------------------------------|
| 1                       | 80.00        | 1.14                            | 78.95          | 98.69                                                |
| 2                       | 1.17         |                                  | 77.59          | 96.99                                                |
| 3                       | 1.16         |                                  | 79.63          | 99.53                                                |
| 4                       | 90.00        | 1.30                            | 90.03          | 100.03                                               |
| 5                       | 1.29         |                                  | 89.34          | 99.27                                                |
| 6                       | 1.31         |                                  | 90.72          | 100.80                                               |
| 7                       | 1.43         |                                  | 99.03          | 99.03                                                |
| 8                       | 1.43         |                                  | 99.03          | 99.03                                                |
| 9                       | 1.44         |                                  | 99.73          | 99.73                                                |
| 10                      | 110.00       | 1.61                            | 111.50         | 101.36                                               |
| 11                      | 1.59         |                                  | 110.11         | 100.10                                               |
| 12                      | 1.60         |                                  | 110.81         | 100.74                                               |
| 13                      | 120.00       | 1.75                            | 121.20         | 101.10                                               |
| 14                      | 1.74         |                                  | 120.50         | 100.42                                               |
| 15                      | 1.74         |                                  | 120.50         | 100.42                                               |
| Mean                    |              |                                  |                | 99.82                                                |
| Relative standard deviation |            |                                  |                | 0.82                                                 |
| Relative confidence interval |          |                                  |                | 0.44                                                 |
| Systematic error        |              |                                  |                | +0.02                                                |
| Statistical insignificance of systematic error δ ≤ Δ_max | 0.01 ≤ 0.47 |                                  |                | Performed                                            |
| Statistical insignificance of systematic error δ ≤ max δ | 0.01 ≤ 0.67 |                                  |                | Performed                                            |

Table 3. Quantitative determination of Amoxicillin capsules 0.5 g results using potassium caroate as analytical reagent

| Day 1 | Day 2 |
|-------|-------|
| Level |       |       |
| 1     | 0.500 | 0.500 |
| 2     | 0.475 | 0.495 |
| 3     | 0.489 | 0.489 |
| 4     | 0.485 | 0.485 |
| 5     | 0.489 | 0.485 |
| Mean  | 0.485 | 0.485 |
| RSD, %| 1.18  | 1.40  |
| δ, %  | 1.01  | 0.41  |
| Mean, g | 0.485 | 0.489 |
| RSD, %| 1.18  | 1.40  |
| δ, %  | 1.01  | 0.41  |
Straight linear dependence is significant in the range of 80–120 % investigated concentrations. The linear equation is used for the calculation of amoxicillin pure substance quantitative determination using reverse iodometric titration. The equation of the calibration curve is $Y = (1.47 \pm 0.15)X$ with a correlation coefficient $r = 0.99$. The obtained results of linearity dependence meet the requirements of the SPhU article for validation of analytical procedures such as titration. The values received correspond to obligatory for evaluation of the proposed procedure.

The limit of detection (LOD) and the limit of quantification (LOQ) are less than 32 % and do not influence the quantitative determination of amoxicillin in the pure substance-using calibration curve method.

The method was successfully applied for the determination of amoxicillin in pure substance for five different concentrations with percent recoveries of 99.82 %. The statistic parameters meet the standards of the requirement investigation.

The procedure of amoxicillin quantitative determination using potassium caroate was performed for amoxicillin capsules. The precision, accuracy, and robustness were determined. Under three concentrations within two days, RSD did not exceed 1.93 % ($\delta = 1.62 \%$).

**Conclusions**

The reaction of Amoxicillin and potassium caroate was studied and the possibility of its application in the pharmaceutical analysis was shown. The developed procedure had statistical results that allow determining the content of Amoxicillin content in pure substance and medicinal preparation. For Amoxicillin in bulk and capsules RSD $= 0.82 \%$, $\delta = 0.44 \%$ and RSD $= 1.93 \%$, $\delta = 1.62 \%$, correspondingly. LOD $= 4.15 \%$, LOQ $= 12.46 \%$. The investigated procedure had statistical results that allow determining the content of Amoxicillin assay using potassium caroate by reverse iodometric titration can be provided into the analysis as an alternative to pharmacopeia.

**Funding**

The work was performed at the Department of Inorganic and Physical Chemistry of the National University of Pharmacy and It is part of the state budget topic “Chemical synthesis, isolation, and analysis of new pharmacologically active substances, establish a link “structure – action”, create new drugs”, state registration number 0198U007011.

**Conflicts of interest:** authors have no conflict of interest to declare.

**Information about authors:**

Serdiukova Yu. Yu., PhD, Associate Professor of the Department of Inorganic and Physical Chemistry, National University of Pharmacy, Kharkiv, Ukraine.

ORCID ID: 0000-0002-4755-3600

Kolinsky O. V., PhD, Associate Professor of the Department of Pharmaceutical Chemistry, National University of Pharmacy, Kharkiv, Ukraine.

ORCID ID: 0000-0003-0558-3164

Tomarovska T. O., PhD, Associate Professor of the Department of Inorganic and Physical Chemistry, National University of Pharmacy, Kharkiv, Ukraine.

ORCID ID: 0000-0003-0045-8209

**References**

[1] Sun, L., Jia, L., Xie, X., Xie, K., Wang, J., Liu, J., Cui, L., Zhang, G., Dai, G., & Wang, J. (2016). Quantitative analysis of amoxicillin, its major metabolites and ampicillin in eggs by liquid chromatography combined with electrospray ionization tandem mass spectrometry. Food Chemistry, 192, 313-318. https://doi.org/10.1016/j.foodchem.2015.07.028

[2] Wang, B., Pang, M., Xie, M., Zhao, M., Xie, X., Zhang, Y., Zhao, X., Wang, Y., Wang, R., Wu, H., Zhang, G., Dai, G., & Wang, J. (2017). Quantitative Analysis of Amoxicillin, Amoxicillin Major Metabolites, and Ampicillin in Chicken Tissues via Ultra-Performance Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry. Food Analytical Methods, 10(10), 3292-3305. https://doi.org/10.1007/s12161-017-0900-8

[3] Blazheyevskiy, M. Y., Karpova, S. P., & Kabachnyy, V. I. (2013). Quantitative determination of some penicillin by iodometric method using potassium peroxymonosulphate. Journal of Chemical and Pharmaceutical Research, 5(11), 637-643.

[4] Pham, T. H. Y., Mai, T. T., Nguyen, H. A., Chu, T. T. H., Vu, T. T. H., & Le, Q. H. (2021). Voltammetric Determination of Amoxicillin Using a
Reduced Graphite Oxide Nanosheet Electrode. *Journal of Analytical Methods in Chemistry*, 2021. https://doi.org/10.1155/2021/8823452

[5] Valenga, M. G. P., Felsner, M. L., de Matos, C. F., de Castro, E. G., & Galli, A. (2020). Development and validation of voltammetric method for determination of amoxicillin in river water. *Analytica Chimica Acta*, 1138, 79-88. https://doi.org/10.1016/j.aca.2020.09.020

[6] Sharma, D. K., Sood, S., & Raj, P. (2019). Spectrophotometric Determination of Amoxicillin, Ampicillin, Cefalexin and Cefadroxil in Pharmaceutical Formulations, Biological Fluids and Spiked Water Samples. *Analytical Chemistry Letters*, 9(3), 345-361. https://doi.org/10.1080/22297928.2019.1644194

[7] Olhman, N. S., & AL-Saffar, R. S. (2015). Spectrophotometric Determination of Amoxicillin in Pharmaceutical Preparations. *International Journal of Enhanced Research in Science Technology & Engineering*, 4(6), 167-173.

[8] Chivulescu, A. I., Badea Doni, M., Cheregi, M. C., & Danet, A. F. (2011). Determination of amoxicillin, ampicillin and penicillin G using a flow injection analysis method with chemiluminescence detection. *Revue Roumaine de Chimie*, 56(3), 247-254.

[9] Fuwei, W., Jinghua, Y., Ping, D., & Shenguang, G. (2010). Molecular imprinting-chemiluminescence sensor for the determination of amoxicillin. *Analytical Letters*, 43(6), 1033-1045. https://doi.org/10.1080/00032710903491104

[10] European Directorate for the Quality of Medicines & Health Care (2010). *European Pharmacopoeia* (Vol. 1-2, 7th ed.) Strasbourg: Council of Europe.

[11] British Pharmacopoeia (2009). London: The Stationery office.

[12] European Pharmacopoeia (2003). (4th ed., pp. 2975-2977). Strasbourg: Council of Europe.

[13] Demskaya, E. V., & Alekseev, V. G. (2005). Analiz lekarstvennykh form ampantsilina, amoksitsilina i tsefaleksina metodom pH-metriceskogo titrovaniya [Analysis of dosage forms of ampicillin, amoxicillin and cephalaxin by pH-metric titration]. *Vestnik Tverskogo gosudarstvennoy universiteta*, 8(2), 177-179. [in Russian].

[14] State Enterprise Ukrainian Scientific Pharmacopoeial Center of Medicines Quality. (2014). *Derzhavna Farmakopeya Ukrayiny* [The State Pharmacopoeia of Ukraine] (Vol. 1, 2nd ed.). Kharkiv: State Enterprise Ukrainian Scientific Pharmacopoeial Center of Medicines Quality. [in Ukrainian].