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ORIGINAL ARTICLE / ÖZGÜN MAKALE

SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR GLICLAZIDE QUANTITATION IN TABLETS

TABLETLERDE GLİKLAZİT MİKTAR TAYİNİ İÇİN SPEKTROFOTOMETRİK YÖNTEM GELİŞTİRME VE DOĞRULAMA

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ABSTRACT

Objective: *A new spectrophotometric method for the gliclazide quantitation in dosage forms has been developed*

Material and Method: *The subjects of the study were modified-release tablets ("Diaglizide" 80 mg, "Diaglizide MR" 60 mg, "Diaglizide MR" 30 mg, "Diabeton" MR 60 mg, "Gliklada" 60 mg). As a reagent, bromocresol green in acetone was used. Analytical equipment: spectrophotometer Specord 200, electronic scales ABT-120-5DM.*

Result and Discussion: *It has been experimentally determined that gliclazide reacts with bromocresol green in acetone medium at room temperature to form a yellow product with maximum absorption at 411 nm. The method was validated for linearity, accuracy, precision, and robustness. The agent's optimal concentration was established and the stability of the investigated solutions was checked by measuring their optical density for 30 minutes. Subordination of Beer's law is observed in the range of 62.00 - 94.00 mg/100 ml. The limit of detection is $4.02 \cdot 10^{-6}$ g/ml, which indicates a high sensitivity of the reaction. The proposed method is validated according to the requirements of the State Pharmacopoeia of Ukraine. The results of the study show that the developed method is simple and affordable to implement and can be used to determine gliclazide in drugs in laboratories for quality control of dosage forms.*

Keywords: *Bromocresol green, gliclazide, spectrophotometry.*

ÖZ

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Amaç: *Dozaj formlarında gliklazid miktar tayini için yeni bir spektrofotometrik yöntem geliştirilmiştir.*

Gereç ve Yöntem: *Gliklazidin oda sıcaklığında aseton ortamında bromokresol yeşili ile reaksiyona girerek 411 nm'de maksimum absorpsiyona sahip sarı bir ürün oluşturduğu deneysel olarak belirlenmiştir. Kimyasal ajanın optimal konsantrasyonu belirlendi ve araştırılan çözeltilerin stabilitesi, 30 dakika boyunca optik yoğunlukları ölçülerek kontrol edildi ve 62.00-94.00 mg/100 ml aralığında Beer yasasına uygun olduğu görüldü. Tespit limiti, reaksiyonun yüksek hassasiyetini gösteren 4.02~10-6 g/ml'dir. Önerilen yöntem, Ukrayna Devlet Farmakopesi gerekliliklerine göre doğrulanmıştır.*

Sonuç ve Tartışma: *Çalışmanın sonuçları, geliştirilen yöntemin uygulamasının basit ve ekonomik olduğunu ve dozaj formlarının kalite kontrolü için laboratuvarlarda ilaçlarda gliklazidin belirlenmesinde kullanılabileceğini göstermektedir.*

Anahtar Kelimeler: *Bromokresol yeşili, gliklazid, spektrofotometri*

INTRODUCTION

Nowadays, diabetes is one of the most common issues. As of 2019, the number of patients with type 2 diabetes was 463 million people. However, according to the IDF, by 2030 the number of patients will increase to 578 million, and by 2045 – to 700 million [1]. Therefore, there is a need to develop new and improved existing methods of hypoglycemic drug analysis.

One of the effective drugs for type 2 diabetes treatment is gliclazide 1-(Hexahydrocyclopenta[c]pyrrol-2(1H)-yl)-3-[(4-methylphenyl)sulfonyl]urea, which is a derivative of sulfonylurea (Figure 1). It lowers plasma glucose levels by stimulating insulin secretion by pancreatic β-cells. Gliclazide-based drugs on the pharmaceutical market are presented in the form of white tablets [2].

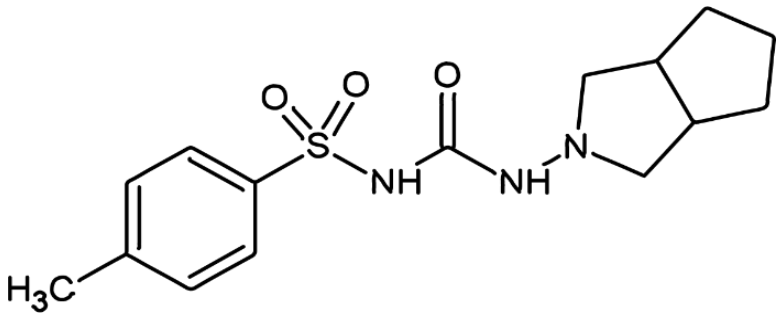

Figure 1. The structure of gliclazide

According to the literature, chromatographic [3-7], mass spectrometric [3, 8], spectrophotometric methods of analysis in UV and visible spectrum [9-12] and indicating-micellar electrokinetic chromatography method [13] are most often used for gliclazide quantitation in dosage forms. However, most of the proposed methods are characterized by either low sensitivity and selectivity, or require expensive equipment or hard-to-reach agents.

Spectrophotometry in the visible spectrum allows you to quickly and very accurately quantify, identify and determine the purity of the substance. According to the requirements of the State Pharmacopoeia of Ukraine, each method must be validated [14]. Therefore, the work aims to develop

and validate a new method for gliclazide quantitation in drugs by absorption spectrophotometry in the visible spectrum.

MATERIAL AND METHOD

The subjects of the study were modified-release tablets "Diaglizide" 80 mg (Farmak (Ukraine) series 111021), "Diaglizide MR" 60 mg (Farmak (Ukraine) series 101120), "Diaglizide MR" 30 mg (Farmak (Ukraine) series 240519), "Diabeton" MR 60 mg (Servier Ukraine LLC series 6019126) "Gliklada" 60 mg (KRKA (Slovenia) series D96697).

The used the following agents and solvent: standard sample solution (SSS) of gliclazide (Jiuzhou, series 2017-0020), bromocresol green (BCG) of analytical grade, acetone of analytical grade.

Equipment: Specord 200 spectrophotometer (Analytik Jena), 1 cm quartz cuvettes, Kern ABT-120-5DM electronic scales and Class A chemical utensils.

Gliclazide determination in general methods

Preparation of the gliclazide reference solution: 0.01950 g of gliclazide is placed in a 25.00 ml volumetric flask, dissolved in acetone and adjusted to the mark with the same solvent.

Preparation of the compensating solution: 1 ml of a 4.2% solution of BCG in acetone is transferred to a 10.00 ml volumetric flask, adjusted to the mark with acetone and mixed.

Transferred an aliquot of gliclazide solution (6.20-9.40 mg) to a 10.00 ml volumetric flask, add 1.00 ml of 4.2% BCG solution and make up to the mark with acetone. Absorption is measured against the background of a compensating solution that does not contain the test substance at a wavelength of 411 nm.

Gliclazide quantitation in tablets

The exact weight of crushed tablets "Diaglizide" 80 mg (0.02860-0.04321 g), "Diaglizide MR" 60 mg (0.07830-0.11847 g), "Diaglizide MR" 30 mg (0.07688-0.11641 g), "Diabeton MR" (0.08323-0.12609) d), "Glyclad" (0.08255-0.12505 g) is transferred into a volumetric flask with a volume of 25.00 ml and adjusted to the mark with acetone. The resulting solutions were stirred and filtered. The first portions of the filtrate are discarded because the first portions of the filtrate were cloudy. We took 1.00 ml of the solution from the filtrate and transfer it to a 10.00 ml flask and add 2.00 ml of BCG solution, make up to volume with acetone and analyze according to the general procedure. In parallel, we carried out the reaction with 1.00 ml of 0.06% SSS gliclazide. The quantitative content of gliclazide in tablets is calculated according to a typical formula.

RESULT AND DISCUSSION

In the course of the experiment, it was found that gliclazide reacts with sulfophthalein dies in acetone to form dyed reaction products (Figure 2).

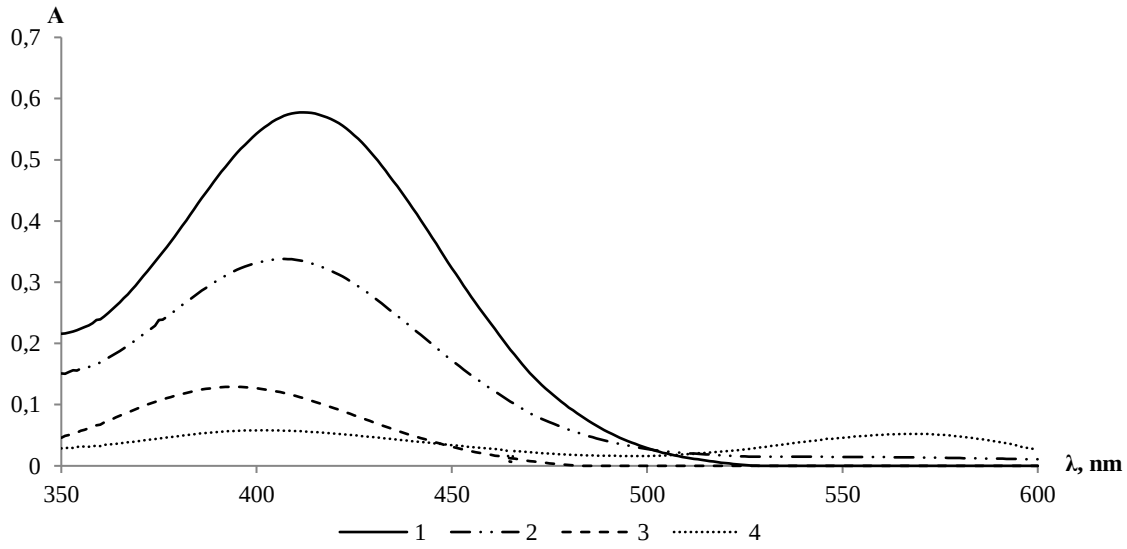

Figure 2. The absorption spectrum of gliclazide reaction products with bromocresol green (1), bromothymol blue (2), bromocresol purple (3), thymol blue (4) in acetone

As can be seen from Figure 2, the highest value of optical density was observed when using bromocresol green. When choosing a solvent, the solubility of gliclazide and BCG, as well as the maximum value of the optical density of the obtained solution, were taken into account. It was experimentally established that gliclazide interacts with BCG in an acetone environment with the formation of a colored product with a light absorption maximum at 411 nm (Figure 3).

It was experimentally determined that BCG interacts with gliclazide rapidly at room temperature and does not require temperature and time adjustment. The limit of detection under such conditions is 4.02 μg/ml.

To determine the ratio of stoichiometric coefficients between gliclazide and BCG, the method of continuous changes (isomolar series method) and the method of molar ratios ("saturation" method) were used.

As can be seen from the Figures 4 and 5, gliclazide interacts with BCG in a ratio of 1:1.

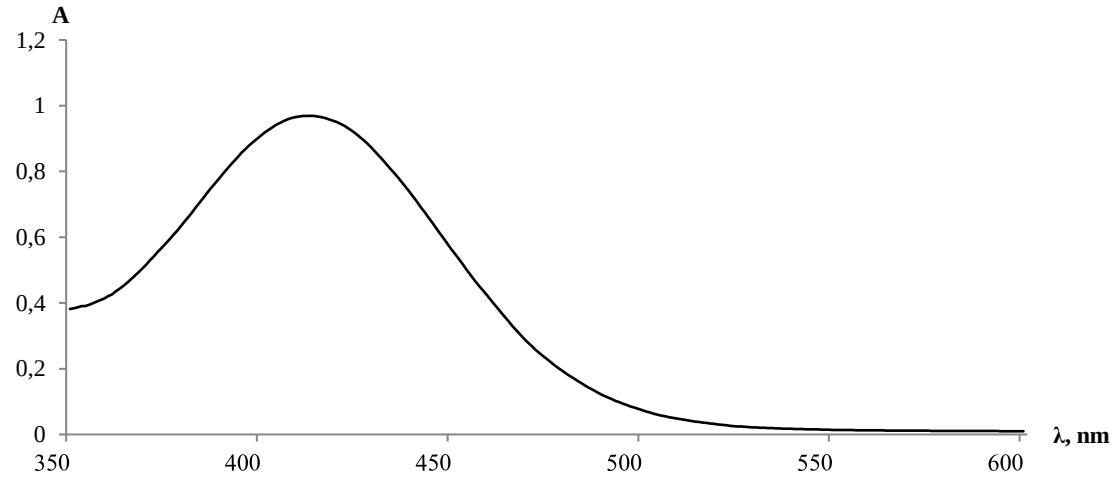

Figure 3. The absorption spectrum of gliclazide reaction product (0.06%) with bromocresol green (4.19%) in acetone

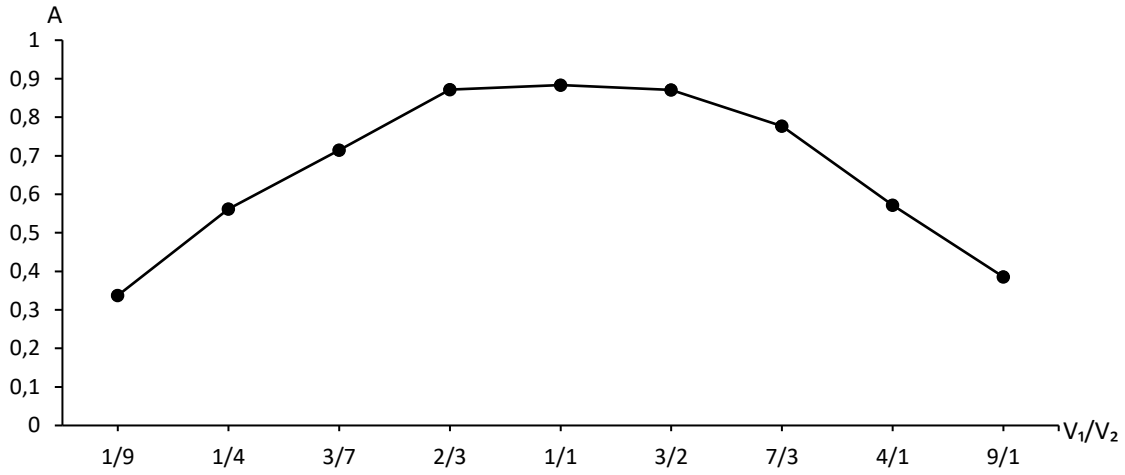

Figure 4. The graph of dependence of absorption on the ratio of components of an isomolar solution (V_1 – volume of 0.01 M BCG solution, V_2 – volume of 0.01 M solution of gliclazide)

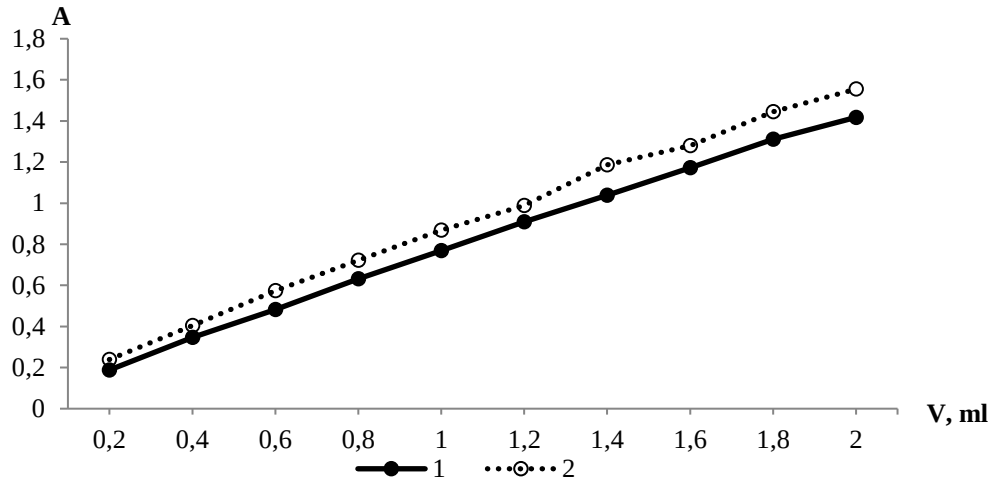

Figure 5. Saturation curves: 1 – gliclazide at constant reagent concentration (1.00 ml of 0.01 M solution); 2 – BCG at a constant concentration of gliclazide (1.00 ml of 0.01 M solution)

Proposed method validation

All validation characteristics of the proposed method are determined by the requirements of the State Pharmacopoeia of Ukraine. Parameters such as robustness, linearity, accuracy and precision were taken into account.

Robustness

The stability of the studied solutions was investigated during the robustness test. To do this, we measured the optical density of the analyzed solution of the corresponding dosage form and the working standard solution of gliclazide every 5 minutes for 30 minutes. As can be seen in Figure 6, the analyzed solution is stable for 30 minutes.

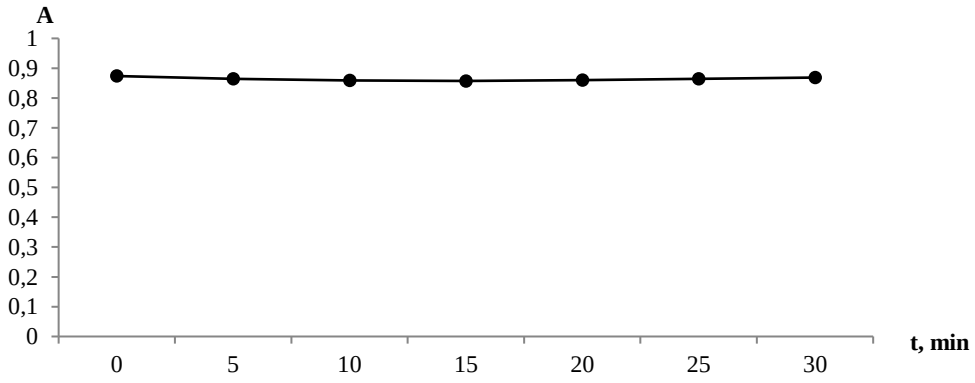

Figure 6. The dependence of the reaction product absorption on time

Linearity

To determine the linearity, 9 measurements of the absorption of gliclazide comparison solution were performed in the range of concentrations in which the subordination of Beer's law is observed, namely 62.00-94.00 mg/100 ml. The curve of absorption dependence on the gliclazide concentration in normalized coordinates is shown in Figure 7.

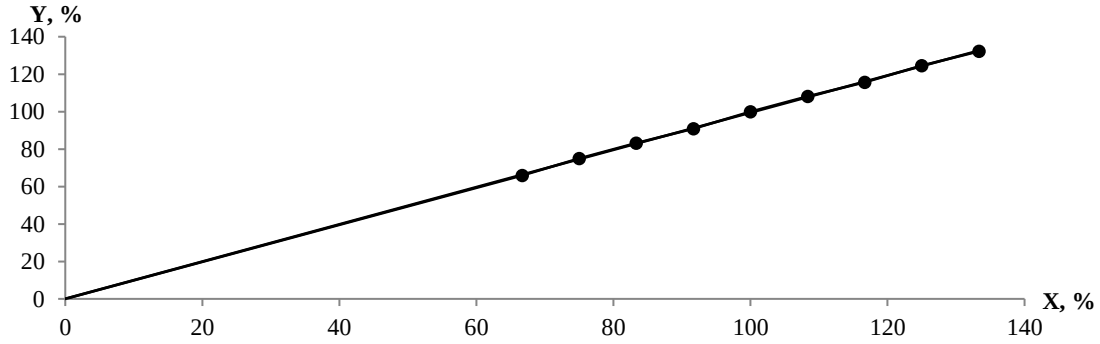

Figure 7. The curve of absorption dependence on the gliclazide concentration (in normalized coordinates) at a wavelength of 411 nm.

The linearity of the proposed method was estimated by regression analysis using the least square method. The obtained values are shown in Table 1.

Table 1. Numerical indicators of linear dependence of the method

Size	Value	Criteria	Conclusion
Equation of linear regression	Yi = bXi + a		
Correlation coefficient, r	0.9998	≥ 0.9962	meets
Residual standard deviation, $S_{x,0}$	0.4355	≤ Δ_{As} (%) /t (95%, 7) 1.689	meets
Intercept term, a ± (S*a*)	0.217 ± (0.685)	≤ t (95%, 7) · S*a* 1.298	meets
Slope, b ± (S*b*)	0.993 ± (0,00670)	—	—

Precision

The precision of the proposed method for each dosage form was determined at the level of convergence. 9 parallel measurements were performed. Three solutions were prepared from three samples, each with three parallel measurements under optimal conditions. In parallel, the absorption of the reference solution was determined and the content of the test substance was calculated. The data obtained are shown in Table 2.

Table 2. Determination of precision and accuracy of the method for the gliclazide quantitation in tablets

Dosage form	$\bar{Z}$ % (n = 9)	S_z%	Δ_{As}	$\Delta_{\%}$	$\delta \leq \Delta_{\%}/3$
Diahlizid, 80 mg	99.70	1.06	1.6	1.97	0.30 ≤ 0.66
Diahlizid MR, 60 mg	100.26	0.88	1.6	1.65	0.26 ≤ 0.55
Diahlizid MR, 30 mg	100.23	0.95	1.6	1.78	0.23 ≤ 0.59
Diabeton MR, 60 mg	99.54	1.25	1.6	2.32	0.46 ≤ 0.77
Gliklada, 60 mg	98.86	0.77	1.6	1.44	0.14 ≤ 0.48

Accuracy

The used the method of additives to establish the accuracy of the proposed method. Different volumes of SSS were added to three equal samples of the drug substance and the optical density was determined three times. As can be seen in Table 3, the results of the determinations are accurate, as the results obtained are within the established confidence interval.

Table 3. Determination of the convergence of the method for gliclazide quantitation in tablets

Dosage form	$\bar{Z}$ % (n = 9)	S_z%	$\Delta_{\%}$	$\lvert 100 - \bar{Z} \rvert$	$\sigma \leq \Delta_{\%}/3$
Diahlizid, 80 mg	99.55	0.85	1.57	0.45	0.45 ≤ 0.52
Diahlizid MR, 60 mg	100.19	0.96	1.78	0.19	0.19 ≤ 0.59
Diahlizid MR, 30 mg	99.94	0.93	1.72	0.06	0.06 ≤ 0.57
Diabeton MR, 60 mg	99.89	0.37	0.68	0.11	0.11 ≤ 0.23
Gliklada, 60 mg	99.65	0.65	1.15	0.35	0.35 ≤ 0.38

Complete uncertainty of analytical methods

To confirm that the developed method will be correctly reproduced in other laboratories, the calculation of the complete uncertainty of the method results was performed. According to the SPU, the projected complete uncertainty of the methodology should not exceed the maximum allowable value.

The forecast of complete uncertainty was calculated by the formula (1):

$$\Delta_{As} = \sqrt{\Delta_{sp}^2 + \Delta_{FAO}^2} \qquad (1)$$

Where Δ_{sp} – the uncertainty of sample preparation;

Δ_{FAO} – projected uncertainty of the final analytical operation.

The calculation of the sample preparation uncertainty of the test solution and the comparison solution is shown in Table 4. The calculations took into account the minimum weight of the finished drug sample.

Table 4. Calculation of the sample preparation uncertainty of the test solution and the comparison solution of gliclazide.

Operation of sample preparation	Uncertainty, %
The investigated solution	
Taking a sample of the finished drug	0.2 mg/28.6 mg · 100 % = 0.70
Bring the volume to the mark in a volumetric flask of 25 ml	0.23
Taking an aliquot of dilution of the finished drug with a pipette per 1 ml	0.74
Bring the volume to the mark in a 10 ml volumetric flask	0.50
The solution of comparison	
Taking a sample of gliclazide	0.2 mg/19.5 mg · 100 % = 1.03
Bring the volume to the mark in a volumetric flask of 25 ml	0.23
Taking an aliquot of dilution of the finished drug with a pipette per 1 ml	0.74
Bring the volume to the mark in a 10 ml volumetric flask	0.50
$\Delta_{sp} = \sqrt{0.70^2 + 0.23^2 + 0.74^2 + 0.50^2 + 1.03^2 + 0.23^2 + 0.74^2 + 0.50^2} = 1.80$	

When performing three parallel measurements with the extraction of the cuvette, the value of the uncertainty of the final analytical operation was equal to 0.70% [15].

Projected complete uncertainty of the analysis results:

$$\Delta_{As} = \sqrt{1.80 + 0.70} = 1.93\%$$

Thus, the projected complete uncertainty of the analysis (1.93%) does not exceed the maximum allowable uncertainty of the method (2.40%) and the method can be reproduced in other laboratories [14].

As a result of the study, a spectrophotometric method for gliclazide quantitation in tablets was developed and validated. The developed method is easy to perform, accessible and meets the requirements of the State Pharmacopoeia of Ukraine, so it can be recommended for gliclazide analysis in laboratories for quality control of drugs.

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AUTHOR CONTRIBUTIONS

Concept: *L.L., S.V.;* Design: *L.L, S.V.;* Control: *L.L, S.V.;* Sources: *L.L, S.V.;* Materials: *L.L, S.V.;* Data Collection and / or Processing: *L.L;* Analysis and / or Interpretation: *L.L;* Literature Review: *L.L;* Manuscript Writing: *L.L, S.V.;* Critical Review: *S.V.;* Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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