Development of a method for the quantitative determination of glibenclamide in tablets

L. H. Leleka*[A,B,D], S. O. Vasiuk[C,E,F]

Zaporizhzhia State Medical and Pharmaceutical University, 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

Aim. To develop and validate a spectrophotometric technique for quantitative determination of glibenclamide in tablets by reaction with 2,3-dichloro-1,4-naphthoquinone.

Materials and methods. In the study, the substance glibenclamide of pharmacopoeial purity was used, tablets – "Maninil" 5 mg and "Glibenclamid-Zdorovye" 5 mg, 2,3-dichloro-1,4-naphthoquinone of the "CFA" qualification was chosen as a reagent, as a solvent – dimethylformamide (DMF) "CFA".

The following analytical equipment was used for the research: spectrophotometer "Specord-200" (Analytic Jena AG, Germany), water bath "MEMMERT WNB7", laboratory electronic scales RADWAG XA 210. 4Y, measuring laboratory vessels of class A.

Results. A new, simple spectrophotometric method of quantitative determination of glibenclamide in tablets by reaction with 2,3-dichloro-1,4-naphthoquinone in DMF medium was developed. The absorption maximum was at 489–491 nm. The value of the detection limit is 10.9 μg/ml, which indicates sufficient sensitivity of the reaction.

Subordination to the basic law of light absorption is within the limits of concentrations of 13.7–27.4 mg/100 ml. In the process of developing the methodology, the following validation characteristics were determined: specificity, linearity, precision, correctness, and robustness.

Conclusions. The methodology for the quantitative determination of glibenclamide was developed and validated according to the requirements of the State Pharmacopoeia of Ukraine. It has been proven that the method is simple, accessible, and validated for such characteristics as linearity, convergence and robustness and can be used for application in laboratories for quality control of medicinal products.

Key words: glibenclamide, 2,3-dichloro-1,4-naphthoquinone, spectrophotometry, validation study, quantitative determination, pharmaceutical preparations, substance, State Pharmacopoeia of Ukraine (SPU).

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Розробка методу кількісного визначення глібенкламіду в таблетках

Л. Г. Лелека, С. О. Васюк

Мета роботи – розробити та валідувати спектрофотометричну методику кількісного визначення глібенкламіду в таблетках за реакцією з 2,3-дихлор-1,4-нафтохіноном.

Матеріали та методи. У дослідженні використали субстанцію глібенкламіду фармакопейної чистоти, таблетки – «Манініл» 5 мг і «Глібенкламід-Здоров'я» 5 мг, як реагент обрали 2,3-дихлор-1,4-нафтохінон кваліфікації «ч. д. а.», як розчинник – диметилформамід (ДМФА) «ч. д. а.».

Для дослідження застосували таке аналітичне обладнання: спектрофотометр «Specord-200» (Analytic Jena AG, Німеччина), водяна баня «MEMMERT WNB7», ваги лабораторні електронні RADWAG XA 210. 4Y, мірний лабораторний посуд класу А.

Результати. Розроблено нову, просту спектрофотометричну методику кількісного визначення глібенкламіду в таблетках за реакцією з 2,3-дихлор-1,4-нафтохіноном у середовищі ДМФА. Максимум поглинання – при 489–491 нм. Значення межі виявлення становить 10,9 мкг/мл, що свідчить про достатню чутливість реакції.

Підпорядкування основному закону світлопоглинання перебуває у межах концентрацій 13,7–27,4 мг/100 мл. У процесі розроблення методики визначили такі валідаційні характеристики: специфічність, лінійність, прецизійність, правильність і робасність.

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Key words: glibenclamide, 2,3-dichloro-1,4-naphthoquinone, spectrophotometry, validation study, quantitative determination, pharmaceutical preparations, substance, State Pharmacopoeia of Ukraine (SPU).

***E-mail:** demyanova0610@gmail.com

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Висновки. Розроблено та валідовано методику кількісного визначення глібенкламіду згідно з вимогами Державної Фармакопеї України. Доведено, що методика є простою та доступною, валідована за такими характеристиками, як лінійність, збіжність і робасність, може бути рекомендована до застосування в лабораторіях з контролю якості лікарських засобів.

Ключові слова: глібенкламід, 2,3-дихлор-1,4-нафтохінон, спектрофотометрія, валідація, кількісне визначення, лікарські засоби, субстанція, Державна Фармакопея України.

Актуальні питання фармацевтичної і медичної науки та практики. 2023. Т. 16, № 2(42). С. 135-140

The problem of type 2 diabetes mellitus is getting bigger every year. According to WHO statistics, the number of diseases will reach the seventh cause of death in the world by 2030 [1]. With this in mind, the pharmaceutical market of hypoglycemic drugs is expanding. Therefore, the search for new, express, selective, and sensitive methods for the quantitative determination of hypoglycemic medications (M) plays an important role at all stages of quality control of finished pharmaceuticals (FP).

For the quantitative determination of the glibenclamide substance, SPU offers alkalimetric titration. 0.400 g of the substance is dissolved when heated in 1.00 ml of 96 % alcohol and titrated with a 0.1 M sodium hydroxide solution to a pink color, using 1.0 ml of phenolphthalein solution as an indicator [2].

Instrumental methods of analysis are most often used to determine glibenclamide as part of medications. Among them, chromatographic and spectrophotometric in the UV and visible range of the spectrum are mainly found. Most often, glibenclamide in tablets and biological fluids is determined by TLC with densitometry [3], high-performance thin-layer chromatography [4,5], liquid chromatography [6], reversed-phase liquid chromatography [7], tandem method of liquid chromatography with mass spectrometry [8], HPLC [9], OF-HPLC [10,11], combined methods of HPLC with spectrophotometry [12,13], spectrophotometric methods [14–16] and UV-visible spectroscopy [17].

A spectrophotometric method for the quantitative determination of glibenclamide by reacting it with 2,3-dichloro-5,6-disiano-1,4-benzochinoin in a buffer solution at pH 2.0 has been previously reported [15]. However, the method requires heating the reaction mixture at 40 °C for 35 minutes, which limits its efficiency. The authors managed to obtain quite high response sensitivity results, but the long heating of the reaction mixture makes this technique long-lasting.

Therefore, development of express, direct, selective, and sensitive spectrophotometric method of quantitative determination of glibenclamide in tablets, certainly, is a promising direction of improvement of quality control of pharmaceutical preparations.

Aim

To develop and validate a spectrophotometric technique for the quantitative determination of glibenclamide in tablets by reaction with 2,3-dichloro-1,4-naphthoquinone.

Materials and methods

All chemicals and reagents used were of analytical or pharmaceutical grade.

The following were used for the research: glibenclamide substance series G-0619012 (MI); tablets "Maninil" 5 mg (BERLIN-CHEMIE MENARINI, Germany) series 18001, "Glibenclamid-Zdorovye" 5 mg (Pharmaceutical company "Zdorovye", Ukraine) series 51121. As a reagent and solvent, 2,3- dichloro-1,4-naphthoquinone and DMF.

Analytical equipment: spectrophotometer "Specord-200" (Analytic Jena AG, Germany), water bath "MEMMERT WNB7", electronic laboratory scales RADWAG XA 210. 4Y electronic laboratory scales RADWAG XA 210. 4Y, measuring laboratory vessels of class A.

General method of determination of glibenclamide

Preparation of the comparative solution of glibenclamide (WRS). Dissolve 0.05150 g of glibenclamide substance in 25.00 ml of DMF in a volumetric flask. If necessary, use an ultrasonic bath. Then fill the flask up to the mark with the same solvent and mix well. Transfer 1.00 ml of the resulting glibenclamide solution to a 10.00 ml volumetric flask, add 1.00 ml of a 0.5 % solution of 2,3-dichloro-1,4-naphthoquinone, and heat the mixture in a water bath at 95 °C for 25 minutes. After the solution has cooled down, fill the flask up to the mark with a DMF solution.

Preparation of the compensation solution. Place 1.00 ml of a 0.5 % solution of 2,3-dichloro-1,4-naphthoquinone in DMF in a 10.00 ml volumetric flask. Heat the flask, fill it up to the mark with the DMF solution, and mix well.

Determination of glibenclamide in tablets

Forty tablets are weighed and ground in a mortar. The exact weight of the obtained tablet mass ("Maninil" 5 mg – 1.23222 g; "Glibenclamid-Zdorovye" 5 mg – 0.99441 g) is placed in a 25.00 ml flask, dissolved, and brought to volume with DMF. The resulting solution is stirred and filtered. The first portions of the filtrate are discarded. From the following portion, 1.00 ml is taken and analyzed according to the proposed procedure. The content of glibenclamide is calculated using the generally accepted formula.

Results

It was experimentally established that glibenclamide reacts with some quinones in a DMF environment to form colored reaction products. The highest optical density value (*Fig. 1*) was observed when using 2,3-dichloro-1,4-naphthoquinone, which has an absorption maximum of 491 nm. Therefore, 2,3-dichloro-1,4-naphthoquinone was chosen as the reagent.

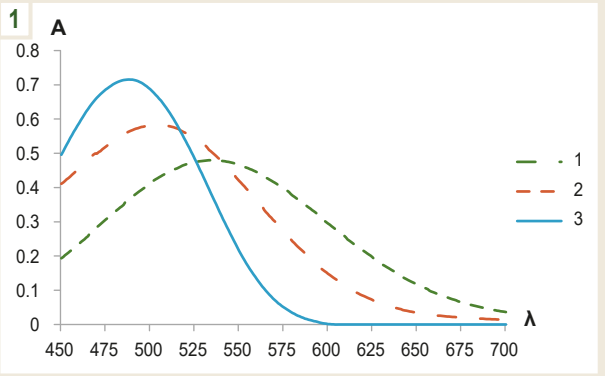
In the process of developing the technique, the optimal conditions for the course of the reaction between glibenclamide and 2,3-dichloro-1,4-naphthoquinone were established.

It was experimentally established that 2,3-dichloro-1,4-naphthoquinone interacts faster with glibenclamide at 95 °C.

Fig. 2 and *3* show that the maximum absorption is observed after 25 minutes of heating. Therefore, a heating time of 25 min was chosen for further tests. The detection limit under such conditions is 10.9 μg/ml.

The concentration of the reagent was determined experimentally by the maximum yield of the reaction product, that is, by the maximum optical density (*Fig. 4*). As can be seen from *Fig. 3*, the maximum value of the optical density is observed at a reagent concentration of 0.5 % in a volume of 1.00 ml, so this concentration was chosen for further work.

The stability of the studied solutions was determined by measuring the optical density every 15 min for 1 h. It was established that the studied solutions are stable for at least 1 hour (*Fig. 5*).



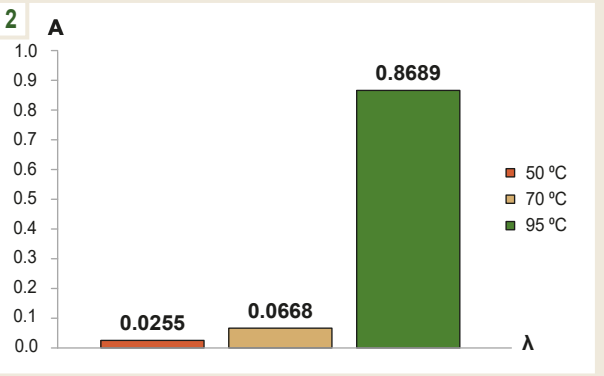
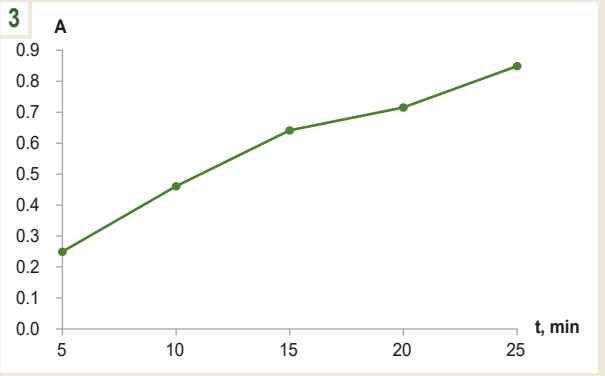


Fig. 1. Absorption spectra of the reaction products of glibenclamide with: 1) 2,5-dichloro-1,4-benzoquinone, 2) p-chloroanil, 3) 2,3-dichloro-1,4-naphthoquinone in DMF.

Fig. 2. Dependence of absorption of glibenclamide reaction products with 2,3-dichloro-1,4-naphthoquinone on the heating temperature of the reaction mixture.



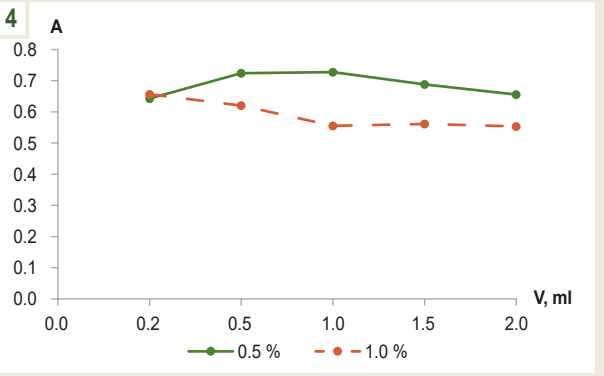
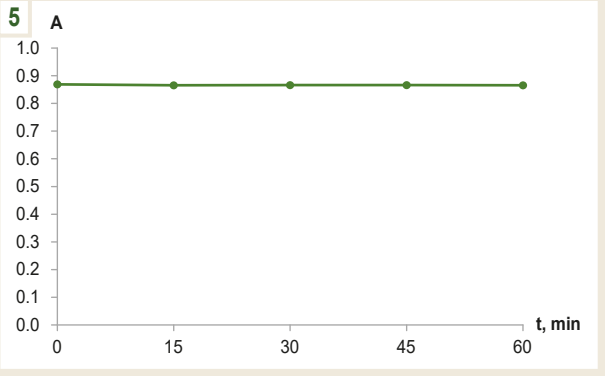


Fig. 3. Dependence of reaction product absorption on heating time.

Fig. 4. Dependence of the absorption of the reaction product on the amount of added reagent.



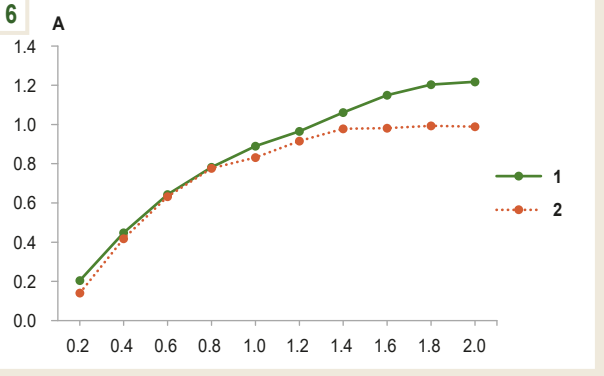
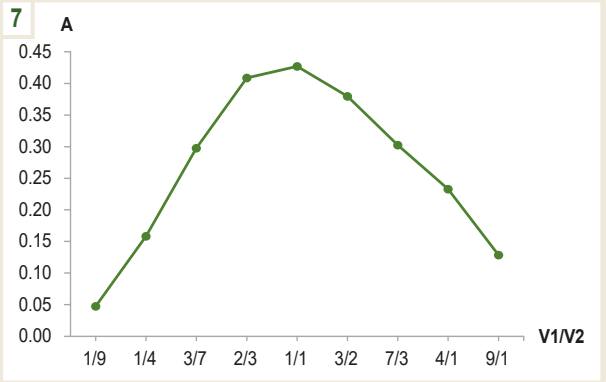


Fig. 5. Graph of the dependence of the reaction product of glibenclamide with 2,3-dichloro-1,4-naphthoquinone in DMF medium on time.

Fig. 6. Saturation curves: **1** – glibenclamide at a constant reagent concentration (1.00 ml of 0.005 M solution); **2** – 2,3-dichloro-1,4-naphthoquinone at a constant concentration of glibenclamide (1.00 ml of 0.005 M solution).



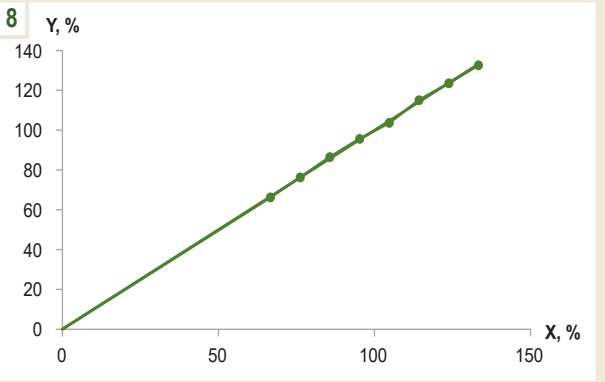


Fig. 7. Graph of dependence of absorption on the ratio of components of an isomolar solution. **V1**: volume of 0.005 M solution of 2,3-dichloro-1,4-naphthoquinone; **V2**: volume of 0.005 M solution of glibenclamide).

Fig. 8. Graph of absorption dependence on glibenclamide concentration (in normalized coordinates) at a wavelength of 491 nm.

Table 1. Numerical indicators of the linear dependence of the technique

Size	Value	Criteria	Conclusion
Equation of linear regression	Yi = bXi + a		
Correlation coefficient, r	0.99961	≥ 0.9631	meets
Residual standard deviation, $S_{x,0}$	0.7032	≤ Δ_{As} (%) /t (95 %, 6) 0.8247	meets
Intercept term, a ± (S*a*)	0.608 ± (1.160)	≤ t (95%, 6) · S*a* 2.245	meets
Slope, b ± (S*b*)	0.994 ± (0.0113)	–	–

To determine the stoichiometric coefficients between glibenclamide and 2,3-dichloro-1,4-naphthoquinone, the method of continuous changes (method of isomolar series) and the method of molar ratios (method of saturation) were used. As can be seen from *Fig. 6* and *7*, glibenclamide interacts with the reagent in a ratio of 1:1.

Linearity. To determine the linearity, 9 absorption measurements of the comparison solution of glibenclamide were carried out in the range of concentrations in which subordination to Beer's law is observed, namely 13.7–27.4 mg/100 ml. The graph of the dependence of absorption on the concentration of glibenclamide in normalized coordinates is shown in *Fig. 8*.

The linearity of the proposed technique was evaluated using least squares regression analysis. The obtained values are given in the *Table 1*.

Precision. The precision of the proposed method for each dosage form was determined at the level of convergence. 9 parallel measurements were carried out. Three solutions were prepared from three measurements, each with three parallel measurements under optimal conditions. In parallel, the absorbance of the comparison solution was determined and the content of the substance under study was calculated. The obtained data are shown in the *Table 2*.

Correctness. To establish the correctness of the proposed methodology, the additive method was used. Different volumes of RSZ were added to three equal samples of the medicinal substance and the optical density was determined three times. As can be seen in *Table 3*, the results of the determinations are correct, since the obtained results are included in the established confidence interval.

Table 2. Determination of the convergence of the results of the quantitative determination of glibenclamide in tablets

Dosage form	$\bar{Z}$% (n = 9)	Sz%	ΔAs	Δ%	δ ≤ Δ% / 3
"Maninil" 5 mg	100.44	0.95	1.6	1.78	0.44 ≤ 0.59
"Glibenclamid-Zdorovye" 5 mg	99.76	0.45	1.6	0.85	0.24 ≤ 0.28

Table 3. Determination of the correctness of the results of the quantitative determination of glibenclamide in tablets

Dosage form	$\bar{Z}$% (n = 9)	Sz%	Δ%	|100-$\bar{Z}$|	σ ≤ Δ% / 3
"Maninil" 5 mg	100.23	0.57	1.06	0.23	0.23 ≤ 0.35
"Glibenclamid-Zdorovye" 5 mg	99.79	0.69	1.29	0.21	0.21 ≤ 0.43

Complete uncertainty of the analytical technique

In order to confirm that the developed method will be correctly replicated in other laboratories, the total uncertainty of the reported method results has been calculated. According to the SPU, the total expected uncertainty in the technique is not expected to exceed the maximum allowable value of Δ_{As}.

Table 4. Calculation of the total uncertainty of the method of quantitative determination of glibenclamide

The operation of sample preparation	Calculation formula parameter	Uncertainty, %
The investigated solution		
Taking a measurement of the finished medicinal product	a_1	0.2 mg/994.4 mg × 100 % = 0.02 %
Bringing the volume up to the mark in a 25 ml volumetric flask	25	0.23
Taking an aliquot of the dilution of the finished medicinal product with a 1 ml pipette	1	0.74
Bringing the volume up to the mark in a 10 ml volumetric flask	10	0.50
Comparison solution		
Taking a dose of glibenclamide	a_0	0.2 mg/51.5 mg × 100 % = 0.39 %
Bringing the volume up to the mark in a 25 ml volumetric flask	25	0.23
Taking a 1 ml aliquot of gliclazide dilution with a pipette	1	0.74
Bringing the volume up to the mark in a 10 ml volumetric flask	10	0.50
$\Delta_{sp}=\sqrt{(0.02^2 + 0.23^2 + 0.74^2 + 0.50^2 + 0.39^2 + 0.23^2 + 0.74^2 + 0.50^2)} = 1.36$ %		

The total uncertainty prediction was computed based on the formula (1):

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

where Δ_{sp} – is the uncertainty of sample preparation;

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

It was taken into account that two solutions are used for the measurement procedure (comparison solution and test solution). When carrying out three parallel measurements with cuvette removal, the uncertainty value of the final analysis operation (Δ_{FAO}) will be equal to 0.70 % [18].

The calculation of the total uncertainty of the method is given in the *Table 4*. The calculations took into account the minimum weight of the finished medication.

Therefore, the total predicted uncertainty of the analysis (1.53 %) does not exceed the maximum permissible uncertainty of the method (3.20 %), which may be replicated in other laboratories [18].

Discussion

During the development of the method, optimal conditions were established for the reaction of glibenclamide with 2,3-dichloro-1,4-naphthoquinone, which involved heating for 25 minutes at a temperature of a boiling water bath in the DMF environment. Under these conditions, the methods for the quantitative determination of glibenclamide in tablets "Maninil" 5 mg and "Glibenclamid-Zdorovye" 5 mg were developed, and validation characteristics such as linearity, precision, correctness, and sensitivity were determined. It has been demonstrated that the developed methods comply with the requirements of the SPU. The predicted total uncertainty of the technique suggests that it can be replicated in other laboratories.

Thus, after analyzing the literature on methods for analyzing glibenclamide in medical forms and biological objects, it can be asserted that the chromatographic methods presented are very accurate and selective [3–7], but they require expensive equipment, lengthy preparation, and costly consumables. Some of these methods require other detection techniques to improve accuracy [12,13].

For spectrophotometric methods in the visible and UV spectrum, there is a need to optimize the conditions for increasing selectivity, such as by using derivatization reagents or changing the pH of the medium [14–16].

Despite the high sensitivity and selectivity of the reported methods, their cost and complexity remain a challenge. Therefore, the development of new, economical, and accessible methods for determining glibenclamide is still necessary.

Conclusions

1. As a result of the conducted research, a spectrophotometric method for the quantitative determination of glibenclamide was developed and validated.

2. The developed method is sensitive, easy to perform, available, and meets the requirements of the State Pharmacopoeia of Ukraine, so it can be recommended for the analysis of glibenclamide in the laboratories of the technical quality control departments of pharmaceuticals.

Conflicts of interest: authors have no conflict of interest to declare.
Конфлікт інтересів: відсутній.

Information about authors:
Leleka L. H., Postgraduate student of the Department of Analytical Chemistry, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0000-0002-3957-6264
Vasiuk S. O., PhD, DSc Professor, Head of the Department of Analytical Chemistry, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0000-0002-1569-9374

Відомості про авторів:
Лелека Л. Г., аспірантка каф. аналітичної хімії, Запорізький державний медико-фармацевтичний університет, Україна.
Васюк С. О., д-р фарм. наук, професор, зав. каф. аналітичної хімії, Запорізький державний медико-фармацевтичний університет, Україна.

References

[1] International Diabetes Federation. (2021). IDF Diabetes Atlas 10th ed. https://diabetesatlas.org

[2] State Enterprise Ukrainian Scientific Pharmacopoeial Center of Medicines Quality. (2015). *Derzhavna Farmakopeya Ukrayiny* [The State Pharmacopoeia of Ukraine (Vol. 1, 2nd ed.)]. Kharkiv: State Enterprise Ukrainian Scientific Pharmacopoeial Center of Medicines Quality. [in Ukrainian].

[3] Andayani, R., Pitasari, F., & Rusdi. (2015). Development and validation of TLC densitometry method for simultaneous determination of metformin HCl and glibenclamide in tablets dosage form. *Journal of Chemical and Pharmaceutical Research*, *7*(9), 159-164.

[4] Bhende, S. D., Varanasi, M. B., & Abbulu, K. (2020). A Sensitive HPTLC Method for the Estimation of Glibenclamide, Rosiglitazone Maleate and Metformin Hydrochloride from a Multicomponent Dosage Form. *Journal of chromatographic science*, *58*(5), 418-426. https://doi.org/10.1093/chromsci/bmz124

[5] Elkady, E. F., El-Zaher, A. A., Elwy, M. H., & Saleh, M. A. (2015). Validated Liquid Chromatographic Method for Simultaneous Determination of Metformin, Pioglitazone, Sitagliptin, Repaglinide, Glibenclamide and Gliclazide – Application for Counterfeit Drug Analysis. *Journal of Analytical & Bioanalytical Techniques*, (S13). https://doi.org/10.4172/2155-9872.s13-007

[6] Havele, S., & Dhaneshwar, S. (2014). Determination of glibenclamide, metformin hydrochloride and rosiglitazone maleate by reversed phase liquid chromatographic technique in tablet dosage form. *Chemical Industry and Chemical Engineering Quarterly*, *20*(1), 39-47. https://doi.org/10.2298/ciceq120607101h

[7] Alam, M. A., Al-Jenoobi, F. I., & Al-Mohizea, A. M. (2018). Rapid, Validated UPLC-MS/MS Method for Determination of Glibenclamide in Rat Plasma. *International Journal of Analytical Chemistry*, *2018*. https://doi.org/10.1155/2018/2569027

[8] Zhang, Y. F., Liu, X. Q., Wang, Y., Xu, X., Zhong, M. K., Zhang, P., & Ma, C. L. (2021). Development and validation of an ultra-high performance liquid chromatography with tandem mass spectrometry method for the simultaneous quantification of direct oral anticoagulants in human plasma. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences*, *1182*, 122952. https://doi.org/10.1016/j.jchromb.2021.122952

[9] Confederat, L. G., Bujor, A., Condurache, I., Profire, L., Miron, A., & Bibire, N. (2021). Development and validation of a high performance liquid chromatography method for simultaneous determination of glibenclamide and lipoic acid. *Farmacia*, *69*(2), 246-252. https://doi.org/10.31925/farmacia.2021.2.8

[10] R. Tengli, A. (2013). Method Development and Validation of Metformine, Pioglitazone and Glibenclamide in Tablet Dosage Form by using RP-HPLC. *Biochemistry & Analytical Biochemistry*, *02*(02). https://doi.org/10.4172/2161-1009.1000130

[11] Haq, N., Alanazi, F. K., Alsarra, I. A., & Shakeel, F. (2014). Rapid Analysis of Glibenclamide Using an Environmentally Benign Stability-Indicating RP-HPLC Method. *Iranian journal of pharmaceutical research : IJPR*, *13*(3), 863-872.

[12] Alhemiary, N. A. F. (2014). Derivative spectrophotometric and HPLC validated methods for simultaneous determination of metformin and glibenclamide in combined dosage form. *Oriental Journal of Chemistry*, *30*(4), 1507-1516. https://doi.org/10.13005/ojc/300408

[13] Porwal, P. K., & Talele, G. S. (2017). Development of validated HPLC-UV method for simultaneous determination of Metformin, Amlodipine, Glibenclamide and Atorvastatin in human plasma and application to protein binding studies. *Bulletin of Faculty of Pharmacy, Cairo University*, *55*(1), 129-139. https://doi.org/10.1016/j.bfopcu.2016.10.002

[14] Sohrabi, M. R., Kamali, N., & Khakpour, M. (2011). Simultaneous spectrophotometric determination of metformin hydrochloride and glibenclamide in binary mixtures using combined discrete and continuous wavelet transforms. *Analytical sciences*, *27*(10), 1037-1041. https://doi.org/10.2116/analsci.27.1037

[15] Gloria, N., & Pius, U. (2018). Spectrophotometric and thermodynamic determination of glibenclamide using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone. *Der pharma chemica*, *10*(3), 9-15.

[16] Antakli, S., Nejem, L., & Alraii, M. (2021). Determination of Glibenclamide By Analytical Spectrophotometry. *Journal of advances in chemistry*, *18*, 40-48. https://doi.org/10.24297/jac.v18i.8963

[17] Patil, S. S., & Bonde, C. G. (2009). Development and validation of analytical method for simultaneous estimation of glibenclamide and metformin HCl in bulk and tablets using UV – Visible spectroscopy. *International Journal of ChemTech Research*, *1*(4), 905-909.

[18] Grizodub, A. I. (2016). *Standartyzovani protsedury validatsii metodyk kontroliu yakosti likarskykh zasobiv* [Standardization of the procedure for validation of methods of quality control of drugs]. State Enterprise "Ukrainian Scientific Pharmacopoeial Center for Drug Quality". [in Ukrainian].