UDC 615.453.6:615.252.349.7].074:543.42.062
DOI: 10.15587/1729-4061.2025.341781

SPECTROPHOTOMETRIC METHOD OF QUANTITATIVE ESTIMATION OF METFORMIN HYDROCHLORIDE IN TABLETS

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In Ukraine, more than one million people suffer from diabetes, and this number increases every year. Metformin is one of the most widely used and most effective hypoglycemic agents for oral administration, available in tablet form with various dosages from multiple manufacturers. In this regard, an important task of modern pharmaceutical analysis is the development of highly accurate, reproducible, accessible, and rapid methods for the quantitative determination of this medicinal product.

***The aim of the work** is to develop a spectrophotometric technique for the quantitative determination of metformin hydrochloride based on the reaction with bromothymol blue in compliance with the SPhU.*

***Material and methods.** As reagent and solvent, bromothymol blue «Honeywell Fluka», acetone of AR grade and purified water were used. Analytical equipment: Specord 200 spectrophotometer, ABT-120-5DM and Radwag XA 210.4Y electronic scales, Elmasonic E 60H ultrasonic bath, measuring glassware of A class.*

***Results**. A novel, sensitive and simple spectrophotometric method for the quantitative determination of metformin hydrochloride (MFH) in tablets was developed and validated. The proposed method is based on the reaction of the analyte with a sulfophthalein dye, in particular with bromothymol blue, using a mixture of distilled water with acetone (1 : 50) as a solvent. The final yellow product has a maximum optical density at 404 nm. Regression analysis of the method demonstrated a strong correlation (not less than 0.998) within the concentration range of 5.20–7.80 μg/mL. Intra-laboratory precision confirmed the absence of interference from excipients and proved the reproducibility of the method within the selected concentration range. The purposed method is accurate, precise and sensitive, so that it is valuable for routine quality control of MFH in solid dosage forms*

***Keywords**: metformin hydrochloride (MFH), quantitative determination, bromothymol blue (BTB), spectrophotometry*

How to cite:
Vasyuk, S., Hidranovich, V., Korzhova, A., Miedviedieva, K., Kucherenko, L., Portna, O. (2025). Spectrophotometric method of quantitative estimation of metformin hydrochloride in tablets. ScienceRise: Pharmaceutical Science, 5 (57), 50–55. http://doi.org/10.15587/1729-4061.2025.341781

1. Introduction

Diabetes is one of the most widespread diseases and is often described as a «disorder of civilization» that related to changes in diet and lifestyle. According to the World Health Organization, 90 per cent of people who suffer from diabetes are diagnosed with the 2-nd type of this disease [1]. For these people, using oral sugar decreasing drugs plays a significant role in maintaining proper life level, normalization of blood glucose concentration and preventing complications such as dyslipidemia, arterial hypertension, blindness and heart attacks.

Metformin hydrochloride (MFH), a 1,1-dimethylbiguanide hydrochloride is a pathogenetic drug of the first line in the pharmacotherapy Type 2 diabetes. Its hypoglycemic action is in virtue of decreasing glucose production by the liver (gluconeogenesis and glycogenolysis), peripheral insulin resistance, inhibition of absorption and rising of glucose recycling in the intestine [2].

Many different methods of analysis have been developed for the quantification of MFH in bulk and dosage forms. There are electrochemical, chromatographic, spectrophotometric, fluorometric and methods of capillary electrophoresis [3–7]. According to the requirements of European, British and Ukrainian Pharmacopoeias the estimation of the concentration of MFH in bulk is carried out with nonaqueous titration, and the quality of drug formulation is checked with UV-spectrophotometry [8–10].

Absorbance at UV-range is susceptible to interference from co-extracted excipients in the tablet formulation [11]. Due to this, derivatization, which leads to the absorption at the visible region, was more appreciated. Additionally, spectrophotometric methods in the visible range are of great interest to us, because of the simplicity, quickness, high accuracy and accessibility of the experiment.

The aim of the presented work was the development and validation of a simple, rapid and economical way of quantitative determination of metformin that differs by high selectivity and reproducibility. In such a way, the spectrophotometric method based on the reaction between MFH with BTB to form a yellow colored derivate was developed.

2. Planning of the research

The following tasks are set, according to the stated purpose:

1. Analysis scientific articles.

2. Investigate the factors that may affect the speed and completeness of the reaction path.

3. Develop the technique for the quantitative determination of metformin and apply it to the analysis of pharmaceutical forms.

4. Validate the spectrophotometric method for determination of metformin in tablets following the requirements of the SPhU.

3. Materials and methods

Objects of study, solvents and equipment.

A reference standard of pure MFH was provided by Harman Finochem Limited. Commercial dosage forms of the drug such as "Diaformin" pills 500 mg from PJSC "Farmak"; "Siofor" pills 500 mg from "Berlin-Chemie" AG; "Methamin" pills 500 mg from LLC "Kusum Pharm"; "Glucophage" pills 500 mg from "Merck Sante" and "Merck, SL"; "Insufor" pills 500 mg from "Ilko Ilac San. ve tic A.S." and "World medicine ilac San. ve tic. A.S."; "Metformin-Teva" pills 500 mg from "Teva Overseas Poland Ltd."; "Metformin Sandoz" pills 500 mg from "Lek S.A." were purchased from the local market.

Analytical equipment: absorbance was measured in 1 cm quartz cuvettes using a double beam ultraviolet-visible spectrophotometer (Analytic Jena, Specord 200). Analytical balances (ABT-120-5DM and Radwag XA 210), an ultrasound bath (Elmasonic E 60H) were also used in the study.

Reagents: a standard solution of BTB (2,56%) was made by adding 2.56 g of pure chemical compound into a volumetric flask and filled with acetone to 100 ml.

Preparation of MFH stock solution.

A standard solution (mg/ml) of MFH was prepared in a measuring flask by dissolving 13.00 mg of pure chemical substance in 4.00 ml of distilled water followed by dilution with acetone to give a final volume of 200.0 ml.

Preparation of MFH sample solution.

A sample of 20 powdered tablets of the selected drug dosage form (containing 500 mg of MFH) nominally equivalent to 81.25 mg of pure MFH was placed, dissolved and made up to 25.00 ml with distilled water in a volumetric flask. After shaking, the content was filtered through a paper filter with a blue stripe (rejecting the first portion of the filtrate).

Then, 1.00 ml of the prepared aqueous solution was transferred to a 50.00 ml flask. Acetone was added to the mark, stirred and filtered again.

Assay procedure.

In a 10.00 ml volumetric flask, the aliquot of the stock solution was transferred with the following adding of 2.00 ml of BTB stock solution. Finally, the received solution was diluted with acetone to the mark and mixed. The measurement of the wavelength of each solution was conducted at 404 nm against a reference solution [12].

Assay validation.

To save time during validation of the method, it was decided to combine the establishing of linearity with accuracy under the recommendations of Hryzodyb O. I. For this purpose, 27 measurements of optical density (3 replicates of 9 concentrations) within the working diapason (80–120% of the nominal concentration of the compound) were performed and evaluated. For reasons of clarity and commonality of experiments, the calculations of statistical data were presented in normalized coordinates [8, 13].

The linearity values were calculated with regression analysis, utilizing the method of least squares (Table 2). For the evaluation of precision, the method of additives was used. This parameter was validated by performing the analysis of drug solutions at 3 concentrations in triplicates was assessed [8, 14].

4. Results

The influence of the following factors was analyzed: the nature and composition of the solvent, the nature and amount of the added reagent, the reaction rate, and the stability of the chemical transformation products over time.

The effect of different solvents on absorbance was studied. The highest absorbance was obtained in DMF, but the reaction product was not stable over time. Ethanol ensured good stability of the reaction product but did not gave acceptable linearity, so it was excluded from further studies. Therefore, an aqueous–acetone medium was chosen for further method development, as it provided stable absorbance values during measurements.

The selection of the optimal reagent during the development of the spectrophotometric method was carried out by comparing the absorption spectra of the reaction products with a twofold excess of sulfonephthalein dyes. The criterion for choosing the indicator was the maximum absorbance of the chemical interaction product. 0.001 M aqueous solution of metformin and 0.002 M acetone solutions of bromothymol blue, bromophenol blue, bromocresol green (BCG), bromocresol purple, cresol red, and thymol blue were investigated as potential reagents. Even though the highest absorbance value was recorded in the reaction of metformin with BCG, BTB was chosen for method development, as it was difficult to establish a linear dependence over a sufficient concentration range when working with bromocresol green.

The next step in developing the new method was to determine the optimal amount of reagent for the reaction. This was experimentally established during the study of linearity, based on the maximum yield of reaction products, and evaluated by the absorbance at the previously selected wavelengths.

MFH containing nitrogen (unshared pair of electrons) in the structure as a primary amino group demonstrates basic properties and interacts with BTB to immediately form the ionic associate with a maximum absorbance at 404 nm (Fig. 1).

The reaction is highly sensitive because the molar absorption coefficient is high, and the detection limit is low. It can be seen in the Table 1.

The method was validated in terms of linearity, accuracy, precision, and robustness according to the requirements of The State Pharmacopoeia of Ukraine (SPhU), particularly to the Article of the SPhU "Statistical analysis of the chemical experiment" [8, 13, 14].

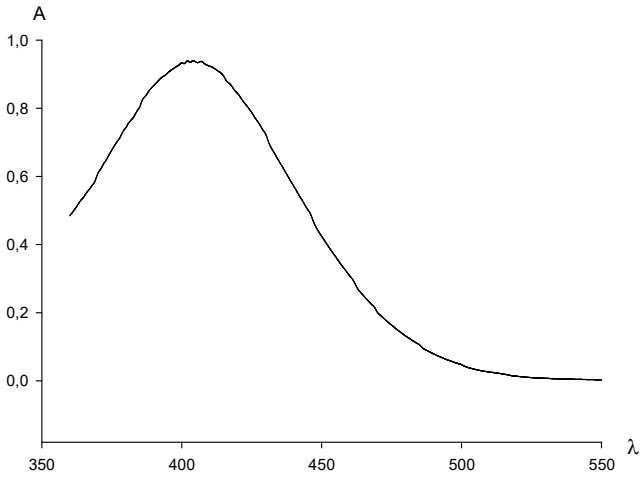

Fig. 1. The VIS absorption spectrum of the result of chemical interaction between MFH (1.00 ml of 0,0065% solution) with BTB (2.00 ml of 2.56% solution)

Table 1

Analytical sensitivity indices

Substance	λ_{max}, nm	ε	a	W_S	C_{min}, μg/ml
MFH	404	$1.95 \cdot 10^4$	0.151	0.0066	0.33

4. 1. Linearity and accuracy

Aliquots of MFH drug formulation (0.80, 0.86, 0.90, 0.96, 1.00, 1.06, 1.10, 1.16 and 1.20 ml) were placed in volumetric flasks. 2.00 ml of the standard BTB solution were added to it, diluted with acetone to 10 ml and mixed. The absorbance was measured at 404 nm using the compensation solution as the background. The analysis of 8 drug formulations was conducted according to this procedure.

A graph of the linear dependence between the concentration and received optical density was drawn (Fig. 2) with the following calculations of basic parameters, which is shown in Tables 3, 4. The linear dependence was constructed in normalized coordinates, and the values of LOD and LOQ were calculated at 2.47% and 7.45%, respectively.

The developed method was accurate, while the one-sided confidence interval (Δ_x) was less than the maximum permissible uncertainty of the analysis (Δ_{As}%). The main values and criteria of linearity and accuracy of tablets are summarized in Table 4.

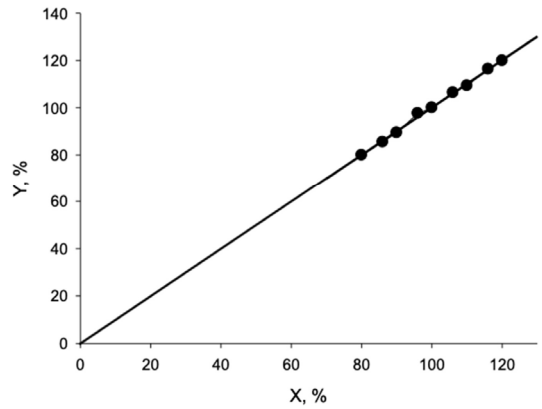

Fig. 2. The plot of the linear correlation of absorbance on the concentration of MFH in the normalized coordinates

Table 2

Range of concentration of MFH and its specific absorption rates in which the Beer's law is obeyed

Substance	λ_{max}, nm	The working range of concentrations, mg/100 ml	$A_{1\,cm}^{1\%}$
MFH	404	0.52–0.78	1,471 ± 15

Table 3

Parameters linear correlation of the pure MFH of the proposed spectrophotometric method

Parameters	Value	Criteria	Conclusion
$b \pm (S_b)$	1.0046 ± ± (0.0194)	–	–
$a \pm (S_a)$	–0.4108 ± ± (1.9649)	$\lvert a \rvert \le \Delta a = t\,(95\%;7) \cdot S_a = 3.72$	satisfied
s_{x0}(%)	0.7520	$\le \Delta_{As}$ (%) / t (95%;7) = 0.92	satisfied
r	0.9987	≥ 0.9977	satisfied

Table 4

Determination of linearity and precision of MFH in tablets with the proposed spectrophotometric method ($n = 9$, $p = 0.95$)

Tablets	Concentration of MFH in tablets	Metrological parameters and criteria				
		$\overline{X}$, %	S_0, %	RSD, %	$\Delta_{x,r}$	Δ_{As}, %
Diaformin	500 mg	98.85	0.6256	0.63	1.15	1.60
Siofor	500 mg	99.49	0.6098	0.69	1.19	1.60
Metamin	500 mg	98.45	0.7207	0.81	1.34	1.60
Glucophage	500 mg	100.4	0.7868	0.79	1.50	1.60
Mefarmil	500 mg	98.55	0.6483	0.74	1.21	1.60
Insufor	500 mg	100.3	0.7002	0.72	1.41	1.60
Metformin-Teva	500 mg	97.59	0.6252	0.62	1.36	1.60
Metformin Sandoz	500 mg	97.38	0.6385	0.64	1.24	1.60

4. 2. Precision

To three equal aliquots of the stock sample solution (0.80 ml) a different volume of the standard stock solution of MFH was added a flask. Thereafter, 2.00 ml of BTB stock solution was added. The resulting solution was then made up to 10.00 ml with acetone.

The developed method is precise, because the systematic error is statistically indistinguishable from zero, which means the deviation of $\overline{Z}$ from 100% does not exceed its confidence interval $\Delta_{\overline{Z}}$. The obtained data is presented in Table 5.

Table 5

Metrological characteristics of the precision

Tablets	ΔZ	RSD	$\Delta_{\overline{Z}}$	$\lvert \overline{Z} - 100 \rvert$
Diaformin	100.7	1.08	2.02	0.70
Siofor	100.3	0.98	1.83	0.3
Metamin	99.75	0.77	1.61	0.25
Glucophage	100.1	0.67	1.25	0.10
Mefarmil	100.6	1.54	2.87	0.60
Insufor	100.3	0.65	1.21	0.30
Metformin-Teva	100.4	0.76	1.42	0.40
Metformin Sandoz	100.5	0.77	1.45	0.50

4. 3. Robustness

In practice, the optical density of the reaction product did not change for at least 30 min (Fig. 3). The insignificant fluctuations in the amount of reagent (± 10%) did not affect the result of the optical density of the determination [8, 13, 14].

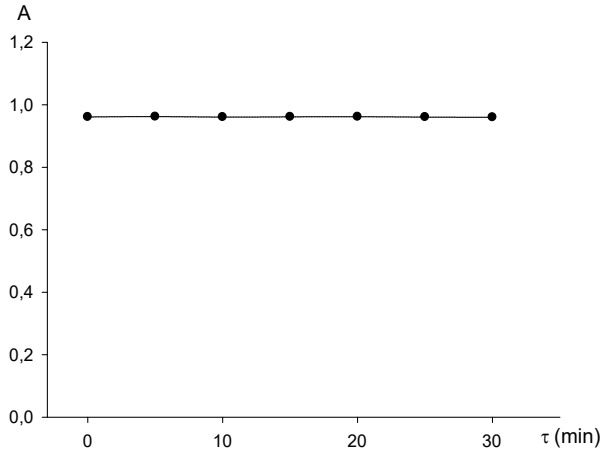

Fig. 3. Effect of time to the absorbance values of the product of the reaction of MFH with BTB, λ_{max} = 404 nm

5. Discussion

A review of the literature on methods for the analysis of metformin in biological samples and pharmaceutical dosage forms demonstrates that spectrophotometric approaches in the UV- and visible regions generally require improved selectivity [11] or are based on reactions that necessitate specific conditions and additional steps, such as extraction or pH adjustment [15, 16]. For instance, one spectrophotometric procedure, similar in both technical principle and analytical outcome, involves dissolving an accurately weighed portion of a metformin tablets in 5 M NaOH, adding hydrogen peroxide solution, heating the mixture in a water bath for 20 minutes, cooling, filtering, and subsequently measuring the absorbance in the visible range at 400 nm [17].

Nevertheless, the relatively low sensitivity (molar absorptivity 1120 l/mol·cm) and prolonged analysis time represent significant drawbacks of this method. The proposed procedure offers enhanced sensitivity (molar absorptivity 1950 l/mol·cm) and provides a more rapid determination of metformin in dosage forms.

The analytical procedure was validated in accordance with the requirements of the State Pharmacopoeia of Ukraine. The linearity equation was established as $Yi = 1.0046Xi + 0.4108$, with a correlation coefficient of $R^2 = 0.9987$. The calibration curve was plotted in normalized coordinates. The outcomes of the accuracy and precision evaluation confirmed accordance with the acceptance criteria. Robustness testing demonstrated that variations of the added reagent (bromothymol blue) within ±10% did not influence the analytical results, and the prepared solutions remained stable for up to 30 minutes.

Although chromatographic methods [18–20] are highly accurate and selective, their extended sample preparation requirements, together with the high cost of instrumentation and consumables, limit their practical accessibility. Therefore, the development of a direct, non-extractive, selective, and sensitive spectrophotometric method for the quantitative determination of metformin in tablets is a promising direction for improving pharmaceutical quality control. However, the exclusive use of aqueous–acetone solutions represent a limitation from the perspective of "green chemistry". Thus, the replacement of such media with water or more environmentally benign solvents, while considering the solubility of the reaction products, would be more rational.

Practical relevance. The new developed method for quantitative determination of metformin can be implemented within the activities of the State Service of Ukraine on Medicines and Drugs Control and may also be applied in the scientific and educational process at the departments of pharmaceutical faculties of higher educational institutions in Ukraine.

Study limitations. The earlier established procedure was validated only for the quantitative assessment of metformin in pharmaceutical preparations. Therefore, it is not advisable to use it without prior research in order to analyze metformin in biological fluids.

Further research prospects. In the future, it is also reasonable to establish the method is the determination of metformin in combined drug products, such as «**Synjardy»** tablets and others. Moreover, it is relevant to introduce the outcomes of this investigation into practical pharmacy and into the activities of the State Service of Ukraine on Medicines and Drugs Control at the regional level.

6. Conclusion

A new visible spectrophotometric method for the quantitative determination of MFH has been developed and validated. It is applicable over a wide range (5.20–7.80 mg/100 ml of metformin) and provides highly accurate and precise results. Several instrumental techniques have been described for the determination of metformin in pharmaceutical formulations; however, they are often time-consuming, involve multiple procedural steps, and require costly instrumentation. The proposed method offers a fast, economical, and sensitive approach and has already been successfully applied to eight tablet dosage forms; therefore, it can be used for the routine analysis of MFH in solid drug formulations.

Conflicts of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial,

personal, authorship or otherwise, that could affect the research and its results presented in this article.

Funding

The study was performed without financial support.

Availability of data

Data will be made available on reasonable request.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies in creating the submitted work.

Acknowledgement

The authors wish to express they're thanks to Zaporizhzhia State Medical and Pharmaceutical University, Ukraine for permission and facilities to carry out the research work.

Authors' contributions

Svitlana Vasyuk: Conceptualization; Supervision; **Vladlena Hidranovich:** Investigation; Writing-original draft; **Alla Korzhova:** Methodology; Software; **Kateryna Miedviedieva:** Validation; Formal analysis; Writing-review & editing; **Liudmyla Kucherenko:** Visualization; Project administration; **Olena Portna:** Resourses; Data curation

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Received 04.09.2025
Received in revised form 13.10.2025
Accepted 18.10.2025
Published 31.10.2025

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