Sensitive Spectrophotometric Method for Metformin Determination in Pharmaceutical Preparations via MBTH–Ferric Chloride Reaction

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This work presents a fast spectrophotometric method for measuring metformin in bulk and pharmaceutical forms. The method uses the oxidative coupling of metformin with 3-methyl-2-benzthiazolinone hydrazone (MBTH) in the presence of ferric chloride, producing a stable green chromophore that absorbs best at 615 nm. Key experimental factors, including reagent levels and addition sequence, were optimized. The calibration curve showed a linear range of 1–16 μg/mL (r = 0.9991), with a molar absorptivity of 3.32×10^4 $L\cdot mol^{-1}\cdot cm^{-1}$. Detection and quantification limits were 0.0089 and 0.040 μg/mL, respectively. Accuracy and precision tests indicated excellent recovery rates (99.87–99.99%) and low RSD (<0.1%). The method successfully applied to pharmaceutical preparations. Compared to existing UV and HPLC methods, this it offers advantages in simplicity, sensitivity, and cost-effectiveness, eliminating the need of pretreatment. This technique may serve as a reliable tool for routine quality control of metformin products as an environmentally friendly alternative.

Keywords: metformin, spectrophotometry, MBTH, ferric chloride, oxidative coupling, pharmaceutical control

Introduction

Metformin is one of the most common oral antihyperglycemic agents for type 2 diabetes mellitus treatment (Fig. 1). Its therapeutic effects include lowering blood glucose levels by suppressing hepatic gluconeogenesis and glycogenolysis, improving insulin sensitivity, and reducing glucose absorption in the intestine [1–3]. Metformin also enhance vascular function, decrease the risk of pancreatic cancer, and reducing hepatic steatosis [3]. It is also used to treat polycystic ovary syndrome (PCOS), further broadening its clinical applications [1,2].

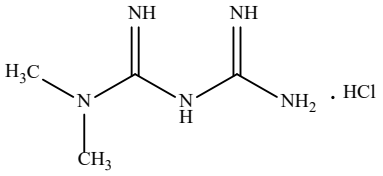

Fig. 1. Chemical structure of metformin hydrochloride.

Given its widespread use, reliable and sensitive analytical methods for quantifying metformin in bulk drug and pharmaceutical dosage forms are crucial for quality control. According to the scientific literature, numerous validated HPLC methods have been reported for the determination of metformin, most of which rely on cation-exchange columns or ion-pair reagents [4–6]. Other analytical techniques, such as capillary electrophoresis [7], ion-selective electrodes [8], conductometric titration [9], flow-injection chemiluminescence [10–12], and adsorptive catalytic square-wave voltammetry [13] are sensitive, but require complex instrumentation and costly reagents. In contrast, only a limited number of spectrophotometric methods have been described for the quantification of metformin hydrochloride in pharmaceutical preparations. The official method listed in the British Pharmacopoeia is direct UV spectrophotometry [14]. Several methods are proposed to improve sensitivity and specificity, including ion-pair complexation with iodide in acetonitrile [15], copper sulfate–catalyzed oxidation in an alkaline cyclohexane medium [16], and ninhydrin [17]. Some of these procedures demonstrate limitations in sensitivity and broader applicability, even though they remain suitable for routine assay and uniformity testing of metformin tablets [17].

The present study introduces an MBTH (3-methyl-2-benzothiazolinone hydrazone)–ferric chloride-based oxidative coupling reaction as the foundation for a new spectrophotometric assay of metformin [18,19]. The method creates a stable green chromophore with high molar absorptivity, providing better sensitivity and

reliability than previously reported spectrophotometric methods [20–27], while remaining simple and low-cost for routine pharmaceutical quality control.

Experimental part

Chemicals and reagents

All chemicals were of analytical reagent grade and used without purification. Ferric chloride ($FeCl_3$), 3-methyl-2-benzthiazolinone hydrazone (MBTH), sodium dihydrogen phosphate (NaH_3PO_4), ammonium hydroxide (NH_4OH), acetonitrile (CH_3CN), and hydrochloric acid (HCl, 1 M) were obtained from commercial suppliers. Pure metformin hydrochloride was provided by SDI (Samarra, Iraq). Distilled water was used in all experiments. Commercial metformin tablet formulations (500 mg and 1000 mg) were purchased from a local pharmacy.

Apparatus

A UV–visible spectrophotometer (Shimadzu UV-1800, Japan) equipped with 1.0 cm quartz cuvettes was used for all absorbance measurements. A digital pH meter (Hanna Instruments, model 300) was used for pH adjustments. Additional equipment included a thermostatic water bath, an ultrasonic cleaner, and an analytical balance (Sartorius BL 210S) with high precision. Standard laboratory glassware, including 10 mL and 100 mL volumetric flasks, was used for solution preparation. Sample handling involved the use of Whatman No. 42 filter paper and a porcelain pestle and mortar.

Preparation of solutions

For buffer solution preparation were mixed 45 mL of 0.025 M NaH_2PO_4, 35 mL of NH_4OH, and 20 mL of CH_3CN. Metformin stock solution (100 μg mL^{-1}) was prepared by dissolving 0.0100 g of pure metformin in 100 mL of the buffer solution. A 0.0616 M $FeCl_3$ solution was prepared by dissolving 1.00 g of $FeCl_3$ in 100 mL of 1 M HCl.

Recommended procedure

For calibration, increasing volumes of the metformin standard solution (100 μg mL^{-1}) were transferred into a series of 10 mL volumetric flasks to obtain final concentrations in the range of 1–16 μg mL^{-1}. To each flask, 2.0 mL of 0.0085 M MBTH solution was added and mixed thoroughly, followed by the addition of 2.0 mL of 0.0616 M $FeCl_3$. The mixtures were allowed to stand for 10 min to ensure complete color development. The volumes were then adjusted to the mark with distilled water. The absorption spectra of the resulting green-colored complex were recorded at 615 nm, consistent with previously reported MBTH-based spectrophotometric methods [18].

Pharmaceutical preparations

Ten commercial metformin tablets were weighed and finely powdered using a porcelain mortar and pestle. A portion of the powdered sample corresponding to 0.10 g (for 500 mg tablets) or 0.12 g (for 1000 mg tablets) was transferred into a 100 mL volumetric flask. The sample was dissolved in the prepared buffer solution, sonicated and shaken for 10 min, and filtered through Whatman No. 42 filter paper to remove insoluble excipients. The filtrate was diluted to volume with distilled water to obtain a stock solution corresponding to the labeled drug content. Appropriate aliquots of this solution were subsequently treated according to the recommended procedure described above.

Results and discussion

Absorption spectrum

The absorption spectrum of the complex was recorded in the range of 400–800 nm against a reagent blank prepared under identical conditions. The absorption spectrum showed a maximum at 615 nm (Figure 1). A slight shoulder was observed near 546 nm, but it did not represent a true absorption maximum. The λmax at 615 nm was chosen as the analytical wavelength fo futher experiments.

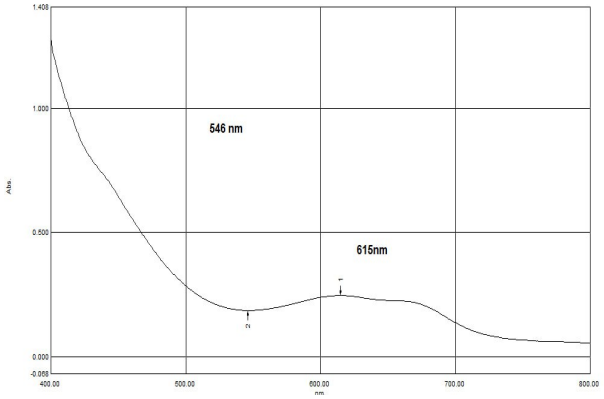

Figure 1. Absorption spectrum of the complex.

Effect of ferric chloride ($FeCl_3$)

The influence of ferric chloride on the absorbance of the metformin–MBTH complex was investigated. The results are presented on Figure 2. Aliquots of 0.5–3.5 mL of $FeCl_3$ solution (0.0616 M) were added to a fixed amount of metformin (1.0 mL of 10 μg mL^{-1}) and 2.0 mL of MBTH under recommended conditions. The maximum absorbance was obtained with 2.0 mL of 0.0147 M $FeCl_3$ solution, beyond which no significant increase in color intensity was observed. This concentration was therefore selected as optimal for all futher experiments.

Effect of MBTH concentration

The influence of MBTH concentration on the absorbance of the metformin–MBTH complex was examined by varying the volume of 0.00855 M MBTH solution in the range of 0.5–3.5 mL, while keeping the drug concentration constant. The absorbance increased with MBTH volume up to 2.0 mL, after which no significant enhancement was observed. Therefore, 2.0 mL was selected as the optimal volume, as it yielded maximum color intensity with minimal blank interference (Figure 3).

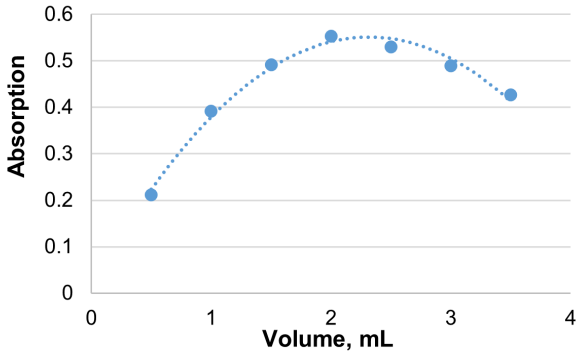

Figure 2. Correlation between the absorbance of the metformin–MBTH complex and the volume of ferric chloride.

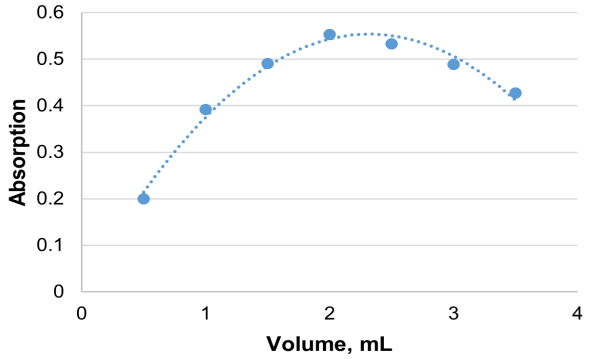

Figure 3. Effect of volumes of MBTH on the absorbance of the metformin–MBTH complex.

Effect of reagent addition order

The influence of the order of reagent addition on the absorbance of the metformin–MBTH complex was systematically evaluated. Among the different sequences tested, the addition order Drug → MBTH → $FeCl_3$ produced the highest absorbance value. This order was therefore selected as optimal for subsequent experiments (Table 1).

Table 1. Effect of reagent addition order on the absorbance of the metformin–MBTH complex.

Order of addition	Absorption
Drug → MBTH → $FeCl_3$	0.525
Drug → $FeCl_3$ → MBTH	0.499
MBTH → $FeCl_3$ → Drug	0.382

Calibration curve and method parameters

A calibration curve for metformin was established in the concentration range of 1–16 μg mL^{-1}. The curve exhibited excellent linearity with a regression equation of y = 0.0436x + 0.1592 and a correlation coefficient (r) of 0.9991, confirming the reliability of the method. The sensitivity of the method was further demonstrated by the calculated limits of detection (LOD, 0.0089 μg mL^{-1}) and quantification (LOQ, 0.040 μg mL^{-1}), as summarized in Table 2.

Table 2. Analytical performance of the proposed spectrophotometric method for metformin determination.

Parameter	Value
Maximum absorption	615 nm
Linearity range	1–16 μg mL^{-1}
Molar absorptivity (ε)	3.32 × 10^4 L mol^{-1} cm^{-1}
Sandell's sensitivity	0.020 μg cm^{-2}
Correlation coefficient (r)	0.9991
Slope	0.0436
Intercept	0.1592
RSD, %	0.58
LOD	0.0089 μg mL^{-1}
LOQ	0.040 μg mL^{-1}

Precision and accuracy

The precision and accuracy of the proposed spectrophotometric method were evaluated by analyzing three different concentrations of metformin (6, 10, and 16 μg mL^{-1}) in triplicate. The obtained results showed relative standard deviation (RSD) values below 2% and recovery values between 99.87% and 99.99%, demonstrating the high repeatability and accuracy of the method (Table 3).

Accuracy and precision were assessed using the following equations:

$$E\% = \frac{O - T}{T} \times 100$$

where *E%* is the relative error, *O* is the observed value, and *T* is the true value [21].

$$SD = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n - 1}}$$

where *SD* is the standard deviation, x_i are the observed values, and *n* is the number of replicates [22].

$$RSD\% = \frac{SD}{\bar{x}} \times 100$$

where *RSD%* is the relative standard deviation and $\bar{x}$ is the mean of replicate values [24].

Application to pharmaceutical formulations

The proposed spectrophotometric method was successfully applied to the assay of commercial metformin tablets (500 mg and 1000 mg). The results demonstrated excellent accuracy, with recovery values ranging from 99.97% to 100.01% and low RSD values, confirming the suitability of the method for pharmaceutical analysis (Table 4).

The developed spectrophotometric method for metformin determination relies on the formation of a green-colored complex produced through the reduction of Fe^{3+} to Fe^{2+} by metformin, followed by oxidative coupling with MBTH. The method was systematically optimized with respect to critical parameters, including $FeCl_3$ and MBTH concentrations as well as the order of reagent addition. The absorption spectrum of the metformin–MBTH complex exhibited a maximum at 615 nm, which was selected as the analytical wavelength. Optimal concentrations of $FeCl_3$ (0.0147 M) and MBTH (0.00855 M) ensured maximum absorbance intensity with minimal blank interference. The method demonstrated excellent linearity over the concentration range of 1–16 μg mL^{-1}, with a correlation coefficient (r) of 0.9991. The low LOD (0.0089 μg mL^{-1}) and LOQ (0.040 μg mL^{-1}) values confirmed the high sensitivity of the procedure. Precision and accuracy, assessed at three different concentration levels in triplicate, showed RSD values below 2% and recovery values between 99.87% and 99.99%, establishing the reliability and reproducibility of the method. Finally, the successful application of the method to commercial tablet formulations with high recovery and low variability highlights its practical utility for routine quality control in the pharmaceutical industry.

Table 3. Precision and accuracy of the proposed spectrophotometric method for metformin determination.

Taken concentration (μg mL^{-1})	Found concentration (μg mL^{-1})	Recovery (%)	Error (%)	RSD (%)
6	6.01	99.99	0.01	0.045
10	10.02	99.98	0.02	0.052
16	15.98	99.87	0.125	0.080

Table 4. Determination of metformin in pharmaceutical formulations using the proposed spectrophotometric method.

Company (Tablet strength)	Taken conc. (μg mL^{-1})	Found conc. (μg mL^{-1})	Recovery (%)	Error (%)	RSD (%)
Pioneer (500 mg)	6	5.92	99.99	0.002	0.035
	10	10.05	99.97	0.025	0.052
	16	15.78	100.01	–0.014	0.071
Merk (1000 mg)	6	6.05	99.99	0.008	0.001
	10	10.07	99.97	0.030	0.053
	16	15.93	100.00	–0.004	0.037

Table 5. Comparison of UV-Visible spectrophotometric methods reported for metformin determination.

Drug form	Analytical method	Experimental conditions / Medium	Linearity range (μg mL^{-1})	LOD (μg mL^{-1})	Ref.
Pure & pharmaceutical prep.	UV-Vis spectrophotometry	Ethanol solvent	2–16	0.021	[24]
Pure & pharmaceutical prep.	UV-Vis spectrophotometry	Distilled water	2–10	1.2	[25]
Pure & pharmaceutical prep.	UV-Vis spectrophotometry	Ninhydrin in alkaline medium (violet chromogen)	8–18	–	[17]
Pure & pharmaceutical prep.	UV-Vis spectrophotometry	0.1 N dilute HCl	1.6–2.72	0.012	[15]
Pure & pharmaceutical prep.	UV-Vis spectrophotometry	Distilled water	20–1000	–	[26]
Pure & pharmaceutical prep.	UV-Vis spectrophotometry	Distilled water	2–12	–	[27]
Pure & pharmaceutical prep.	UV-Vis spectrophotometry (proposed)	Oxidative coupling of metformin with MBTH (green complex)	1–16	0.0089	This study

Conclusion

A novel spectrophotometric method has been developed for the determination of metformin in pure and pharmaceutical forms. The procedure is based on the reduction of Fe^{3+} by metformin, followed by oxidative coupling with MBTH with formaton of stable green complex with maximum absorbance at 615 nm. The method demonstrates excellent analytical characteristics, high linearity (1–16 μg mL^{-1}, r = 0.9991), low detection (0.0089 μg mL^{-1}) and quantification limits (0.040 μg mL^{-1}), good precision and accuracy (recoveries of 99.87–99.99% with RSD <2%). Unlike conventional chromatographic the proposed method does not require extraction, derivatization, or pre-treatment steps. The successful application to commercial formulations confirms this spectrophotometric approach offers a simple, rapid, sensitive, and cost-effective alternative for routine quality control of metformin in pharmaceutical industries.

Study limitations

In this study, several limitations need to be acknowledged. The study used a limited range of commercial metformin formulations, which may not cover all the variations present in the market. In addition, environmental factors such as temperature and humidity could interfere with the stability of the metformin complex. The method also involves manual steps, which brings the possibility of human error and might affect the reproducibility of the results in a less controlled environment. Finally, the validation was conducted in a single lab, and more research center validations would be necessary to confirm the robustness and applicability of the method.

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