American Journal of Biomedicine and Pharmacy
https://biojournals.us/index.php/AJBP
ISSN: 2997-7177

Spectrophotometric Determination of Metformin via Schiff Base Formation with 2-Hydroxy-1-Naphthaldehyde

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Received: 2026, 04, Feb
Accepted: 2026, 10, Mar
Published: 2026, 20, Apr

Annotation: A sensitive and straightforward spectrophotometric technique for measuring metformin levels was established in this work. The process is reacting 2-hydroxy-1-naphthaldehyde in an acidic solution to create a Schiff base, which has a high absorbance at 453 nm and an orange-yellow hue that indicates Schiff base production. The calibration curve validated the quantitative approach by demonstrating a linear relationship for values between 2 and 23 µg/mL, as indicated by the correlation coefficient R^2 = 0.9995. The method's great sensitivity and accuracy were demonstrated by the molar absorbance of 3693.976 L/mol/cm² and the Sandel index sensitivity of 0.0767 µg/cm² with a recovery rate of 100.53%. With a standard deviation of 0.0479%, the accuracy and repeatability were good. Additionally, the quantification limit was 0.098 µg/mL and the detection limit was 0.032 µg/mL. This approach is thought to be extremely sensitive to the medication. Because the ratio between the reactant and the medication was 1:1, the correctness of the standard technique was checked using the Job approach and the suggested chemically equivalent molar ratio. The concentration of metformin in the pharmaceutical formulation was satisfactorily established using this approach.

Keywords: Schiff base, metformin, 2-hydroxy-1-naphthaldehyde, spectrophotometry.

1. Introduction

Diabetes is a metabolic condition that manifests as elevated blood glucose levels caused by either insufficient insulin production or insulin resistance. Type 1 diabetes is characterized by the destruction of β-cells in the pancreas, whereas type 2 diabetes may be caused by insufficient β-cells [1]. The American Diabetes Association and European Association recommend metformin as the first line of therapy for type 2 diabetes [2]. Up until 2009, type 1 diabetes affected 21% of young people in the United States, and its annual prevalence exceeded 3% worldwide [3]. 4.2 million people with diabetes passed away in 2019 [4].the high death rate, which is mostly caused by metabolic and cardiovascular problems [5]. Even if metformin is the best monotherapy for those with type 2 diabetes, di- or tri-therapy must be recommended if blood glucose levels aren't controlled. For glycemic control, prevention of adverse effects from the maximal dose, and induced efficacy, binary therapy should be administered. The improvement of glucose metabolism is the primary goal of administering linagliptin (LNT) with metformin HCl (MTF), as well as because of their safety, particularly in the elderly, and their effectiveness in treating chronic type 2 diabetes [6]. Metformin-intolerant patients should be treated with combination therapy of LNT and MTF because co-administration of LNT and MTF has numerous advantages for diabetic patients, including reducing the risk of hypoglycemia. Additionally, a small dose of MTF (500 mg/12 hr) combined with LNT as a single dosage form is more effective than a maximum dose of MTF (1000 mg/12 hr) alone [7]. Metformin and linagliptin may be quantified using a variety of chromatographic techniques [8], [9], [10], [11], [12], and [13]. For the analysis of metformin and linagliptin, several spectrophotometric methods have been reported [14], [15], [16], [17], [18], [19], [20], and [21].The British Pharmacopoeia lists 1-cyanoguanidine (CYG) as an official impurity for metformin hydrochloride [22]. CYG is regarded as a chemical that causes skin irritation, mutagenesis, and cancer [23].

2. Materials and Methods:

2.1. Used devices: The UV-Vis spectroscopy investigation was conducted using a device from PG Instruments Ltd. that included an MS300HS heating plate, a Kern 770GS/GJ model BL210 balance, and a Shimazu 1800 dual-beam spectrometer with a 1 cm quartz cell.

2.2. Substances and solutions: The purified materials used in this study were provided by Avonchem (UK) and FLUKA (USA). The solutions contained distilled water and ethanol as solvents. We purchased metformin from Samarra Pharmaceutical Company.

2.3. solution of metformin

0.1 g of metformin was dissolved in distilled water to create a 1000 µg/ml metformin solution. The same solvent was then used to add the solution to 100 mL of a volumetric flask to the necessary volume. After that, the solution was diluted to 100 µg/mL. Ten milliliters of this diluted solution were then combined with one hundred milliliters of distilled water.

2.4. Reagent solution 0.01 M

To prepare this solution, 0.172 g of 2-hydroxy-1-phthalide powder was dissolved in a specific amount of ethanol. Distilled water was then added to fill a 100 mL volumetric flask to the appropriate level.

2.5. Hydrochloric acid solution 1 M

Hydrochloric acid was diluted in a volumetric flask containing distilled water by adding 8.3 mL of the acid (12.77 M). The solution was then brought to the required level by adding the same solvent.

Afterward, 5 mL of the solution was taken and diluted in 100 mL of distilled water to obtain a concentration of 0.05 M.

2.6. Pharmaceutical Solution 250 µg/mL

Take 1 tablet, each containing 500 mg of the active ingredient. The pharmaceutical formulation contains Glucophage at a concentration of 500 mg. We take 25 mg in a 100 ml volumetric bottle to obtain a solution with a concentration of 250 micrograms/ml.

3. Results and discussions

The method is based on adding the reagent solution 2-hydroxy-1-naphthaaldehyde to the drug in an acidic medium, which leads to the formation of an orange-yellow product that gives the highest absorption at 453 nanometers. [24].

Effect of Reagent Volume

In a 20 mL volumetric flask, different volumes of reagent at a concentration of 0.01 M and acid HCl at a concentration of 1 M in a volume of 1 ml. Then, After that, 1.5 ml of the drug at a concentration of 250 µg/mL, and distilled water was added to reach the required volume. It may be noted that the optimal volume of reagent that was added, 1 ml, had the highest absorption, as shown in Table 1.

Table 1. Effect of 2-Hydroxy-1-nifthaldehyd volume.

Reagent volume mL	Absorbance
0.6	0.514
0.8	0.572
1	0.650
1.2	0.598
1.4	0.570
1.6	0.576

Effect of temperature:

To determine the effect of temperature on absorption, temperatures from 10-35 degrees Celsius were taken, as the temperature of 25 C˚ gave the highest colored absorption, so it was adopted in the rest of the experiments, as in Table 2

Table 2. Effect of temperature change.

Temperature	Absorbance
10	0.402
15	0.535
20	0.648
25	0.652
30	0.653
35	0.654

Effect of HCl acid

By adding different volumes of 0.05 M acid to determine which volume gives the best absorption of the acid, and from the results it can be determined that the 1 mL volume has the best absorption of the colored substance, as shown in Table 3.

Table 3. Effect of HCl acid volume.

HCl volume mL	Absorbance
0.5	0.571

1	0.652
1.5	0.64
2	0.598

Effect of Coupling time:

To determine the optimal Coupling time, different time intervals were used. The results showed that the optimal time was 10 minutes, as shown in Table 4.

Table 4. Effect of Coupling time.

Coupling time	Absorbance
Beginning	0.613
5	0.641
10	0.653
15	0.653
20	0.653

Estimated output duration: The output remained stable for a full hour overnight.

Final Absorption Spectrum

After adding 1.5 mL of metformin at a concentration of 250 µg/mL, 1.0 mL of 2-hydroxy-1-naphthalide reagent at a concentration of 1×10^{-2}, and 1 mL of 1.0 M hydrochloric acid, optimal conditions were achieved. The volume was then completed to the mark in a 20 mL volumetric flask with distilled water. The final absorption spectra of the orange-yellow product were compared with those of the sham solution. It was found that the product exhibited maximum absorption at a wavelength of 453 nm, while the sham solution showed no absorption in this region., as shown in Figure 1

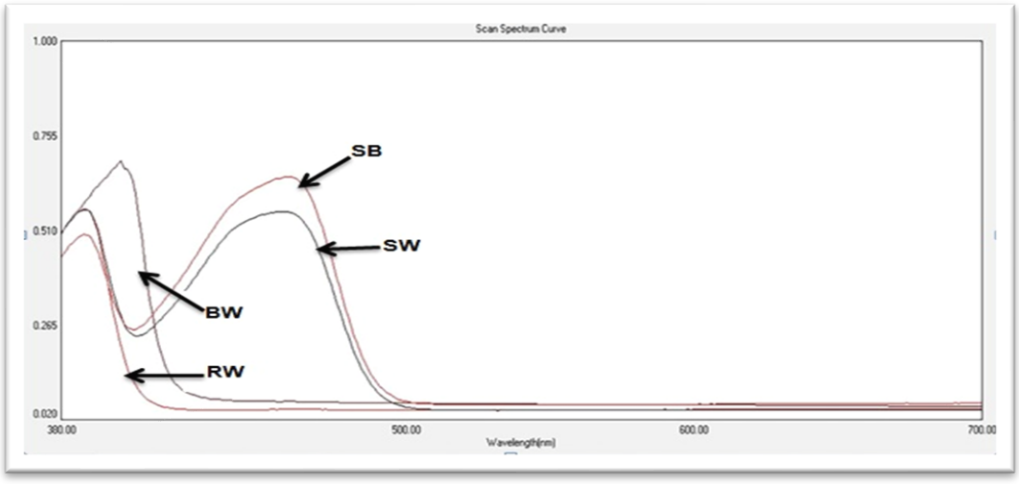


Figure 1. shows the absorption spectrum of the drug versus the blank solution Sb, the drug versus water Sw, and the sham solution with water bw.

Calibration Curve

One milliliter of the reagent and one milliliter of HCl were placed in several 20 milliliter volumetric flasks. Each container was then filled with varying amounts of metformin, ranging from 0.5 to 4.5 mL, and allowed to stand for 15 minutes before being diluted with distilled water to the necessary

level. After measuring each bottle's absorbance, the correlation coefficient was determined to be $R^2 = 0.9995$, with a molar absorption coefficient of 3693.976 L. mol-1. Cm-1, as seen in Figure 2.

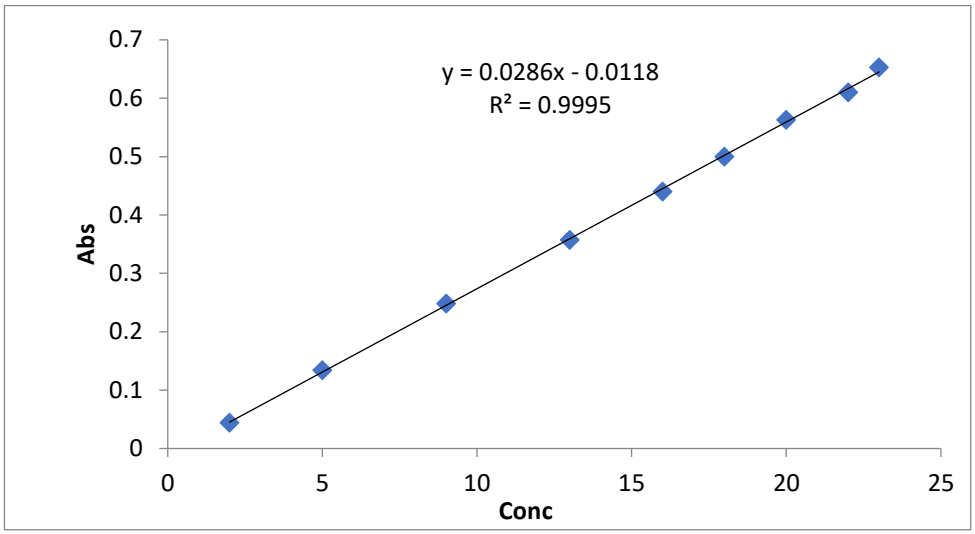


Figure 2. Calibration curve for metformin.

Accuracy and Precision

The relative standard deviation (RSD) was determined to be 0.0479, suggesting high repeatability, by calculating the relative standard deviation and relative error based on three metformin concentrations and computing the arithmetic mean of repeated measurements under ideal conditions. The Sandell sensitivity was 0.0767µg/cm-2, indicating strong sensitivity, while the recovery value was 100.53%. The method's sensitivity was measured with a limit of quantitative measurement (LOQ) of 0.098 µg/mL and a limit of detection (LOD) of 0.032 µg/mL. These outcomes show how accurate the approach is. [24,25]. The calculated results are consistent, as shown in Table 5.

Table 5. Accuracy and Precisionof the proposed method.

conc. Of D taken	Found conc. Of D	Recovery %	Average of Recovery %	RSD%
5	5.097	101.94		0.015
13	12.89	99.15	100.53	0.079
20	20.1	100.50		0.0497

Continuous Change Method

Different volumetric bottles of 20 ml each were filled with increasing volumes of the drug (0.5-4.5 ml) and decreasing volumes of the reagent (0.5-4.5 ml) using Job's method and the molar ratio to determine the equivalency of the substance with the reagent and drug correlation coefficient at 250 ppm. One milliliter of HCl per bottle. The necessary volume was subsequently reached by adding distilled water [26, 27]. It was discovered that the drug-reagent ratio was 1:1., as shown in Figure 3.

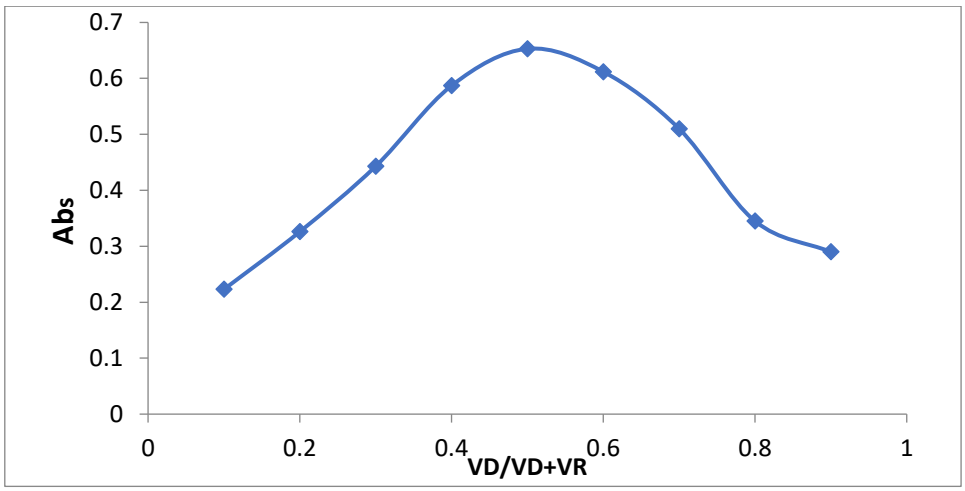


Figure 3. Job's plot for determining the stoichiometric ratio between metformin and the reagent.

While the drug's volume was constant at 1.5 ml of its 250 ppm concentration, several quantities of the reagent (0.5–4 ml) were utilized to calculate the molar ratio. Similar to Job, the molar quantities were put into several 20 ml volumetric vials. Add one milliliter of HCl to each bottle. The necessary volume was then reached by adding distilled water [28]. It was discovered that the medication and reagent had a 1:1 ratio, as in Figure 4.

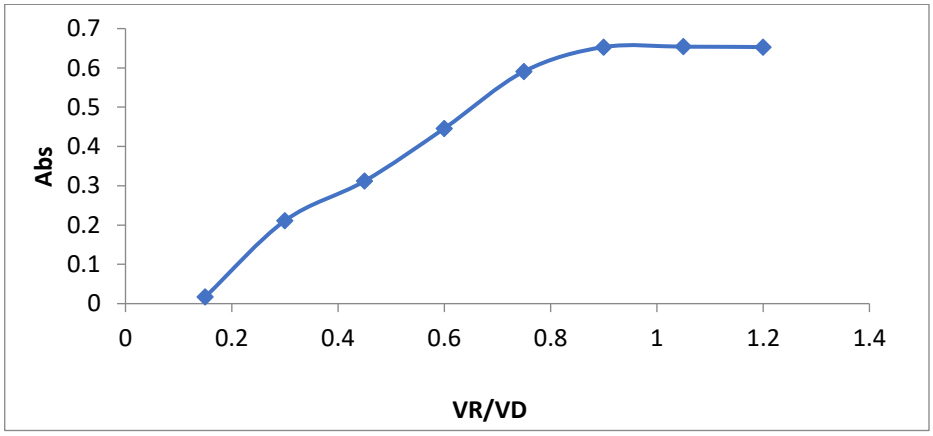
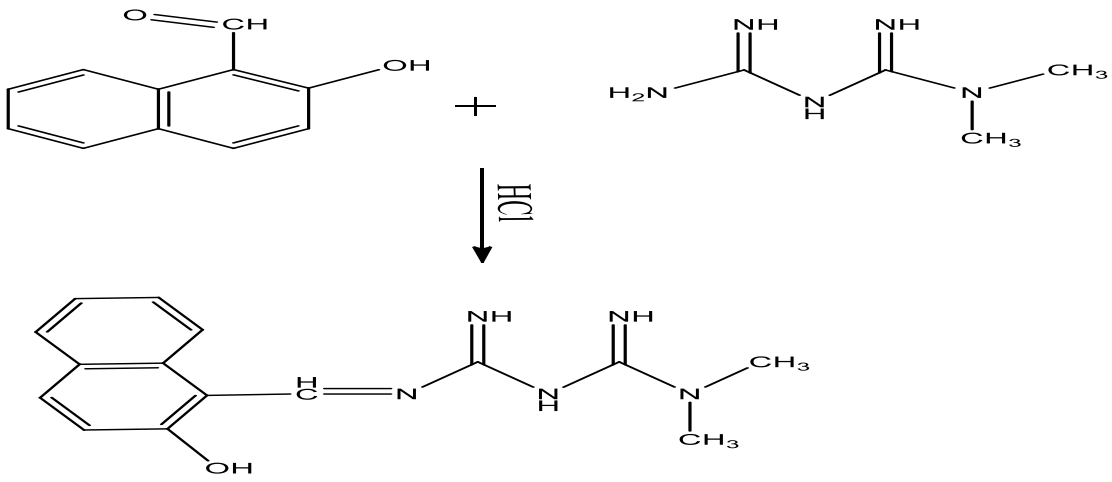


Figure 4. Mole ratio plot for determining the stoichiometric ratio between metformin and the reagent.

The proposed chemical equation for this method [29]



Pharmaceutical solution 250 micrograms/ml:

In a volumetric flask containing 100 mg of the pharmaceutical solution, equivalent to 0.1000 g of metformin, and mixed in the same manner as for the standard solution, 25 mL of this solution was taken and the volume was filled to the mark in a 100 mL volumetric flask. To obtain a 250 µg/mL solution, and to confirm the amount of metformin in the pharmaceutical preparation, three concentrations of the solution were extracted; the results are shown in the table 6.

Table 6. Application of the proposed method for drug estimation in pharmaceutical preparations.

	Taken µg/ml	**Obtained µg/ml**	**Rec.%**
pharmaceutical preparation	5	5.08	101.17
	13	12.50	103.12
	20	20.13	100.5

4. Conclusions:

Metformin content was measured using the Schiff base-forming reaction in an acidic medium with 2-hydroxy-1-phthaldheide reagent at a concentration of 1×10^{-2}. The result was an orange-yellow substance with a maximum absorbance at 453 nm in wavelength. The Sandel sensitivity was 0.0767 µg/cm², the estimate factor was 0.9995, and the molar absorbance was 3693.97 L/mol•cm². It followed Beer's law at concentrations between 2 and 23 µg/mL. The relative standard deviation was less than 0.0479%, and the limit of detection was 0.032 µg/mL. This method was accurate and worked nicely. The process was simple, the reaction was finished, and the concentration stabilized in ten minutes and stayed that way for almost forty.The proposed technique has been effectively used for the determination of metformin in pharmaceutical preparations.

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