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RESEARCH ARTICLE OPEN ACCESS

Development of a spectroscopic approach for the estimation of glimepiride by oxidation reaction in an acidic environment and measurement of the area under the absorption curve using the UV-visible technique

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Abstract
Objective: This study aimed to develop and validate a simple spectrophotometric method for the determination of glimepiride (GLM) in pharmaceutical tablets, based on the formation of a colored complex with potassium permanganate in an acidic medium. Both direct absorbance measurement at the λ_{max} of the complex and the Under Absorption Curve (UAC) approach were applied to enhance reliability and sensitivity
Method: All experimental conditions were optimised to achieve the best analytical response. The area under the absorption curve (UAC) was measured for both the pure drug and the colourimetric reaction product as a supporting tool for quantitative estimation.
Results: The method demonstrated good linearity across a range of concentrations with high precision from (0.5–15) µg/mL, linearity was (R^2=0.997), low detection limits (LOD = 0.166) µg/mL, and scientifically acceptable recovery rates Rec % (101.291). The colourimetric reaction had a higher absorbance at λ_{max} (400nm) and a significantly larger area under the absorption curve (UAC) of 33.656, confirming the occurrence of the chemical reaction and the formation of a coloured product responsible for the absorption.
Conclusion: The proposed spectrophotometric method, using either direct absorbance at λmax or UAC determination, provides a precise, sensitive, and economical procedure suitable for routine quality control of GLM in pharmaceutical formulation.

Keywords: GLM, UAC, Pharmaceutical tablet, UV–Visible spectrophotometer, Method validation

Plain English Summary

Glimepiride is a medicine used to control blood sugar in people with type 2 diabetes. Usually, laboratories use expensive machines to measure this drug in tablets. In our work, we developed a simple and low-cost method that uses light to detect the medicine after a colour reaction. Instead of measuring the colour at only one point, we measured the entire curve of the colour change (called the area under the absorption curve, or UAC). This gave more sensitive and reliable results. The method could help laboratories without advanced equipment to test medicines accurately.

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Introduction

Glimepiride (GLM) is an important antidiabetic drug widely used as an oral hypoglycaemic agent for the treatment of type 2 diabetes mellitus. GLM appears as a white to yellowish-white crystalline powder, practically insoluble in water. Its IUPAC name is 1-[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl]phenyl]sulfonyl]-3-(trans-4-methylcyclohexyl)urea, with the molecular formula $C_{24}H_{34}N_4O_5S$ and a molecular weight of 490.62 g/mol. Figure 1 shows the formula structure of the drug. Accurate determination of GLM in pharmaceutical formulations is essential to ensure drug quality and therapeutic efficacy.

Several analytical techniques, such as High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometry (LC-MS), have been reported for the determination of GLM. While highly sensitive, these techniques are expensive, time-consuming, and require sophisticated instrumentation, making them less suitable for routine quality control, particularly in laboratories with limited resources. (UV-VIS) Spectrophotometry provides a simpler and more economical alternative for pharmaceutical analysis. The UAC approach offers added advantages over single-wavelength UV-Vis methods. Instead of measuring absorbance at a single wavelength (λmax), UAC determines the area under the absorption curve between two selected wavelengths. This improves accuracy, reduces the effect of spectral noise or minor wavelength shifts, and enhances the reliability of the measurement. In this study, a UAC-based spectrophotometric method was developed and validated for the quantification of GLM in tablet formulations. The method was assessed for linearity, precision, accuracy, and robustness, demonstrating that UAC can be effectively applied as a refined spectrophotometric approach for routine quality control of GLM. According to a review of relevant literature, UV-VIS spectrophotometry (1, 2, 3, 4, 5, 6, 7, 8, 9), Rc-Hplc (10, 11, 11, 13) liquid chromatography (13, 15), TLC (16), LC-MS/MS (17), UPLC-MS/MS (18), MIP (19). Other advanced techniques include Capillary Electrophoresis (CE) and electrochemical methods, which use modified electrodes to detect glimepiride based on redox behaviour (20, 21).

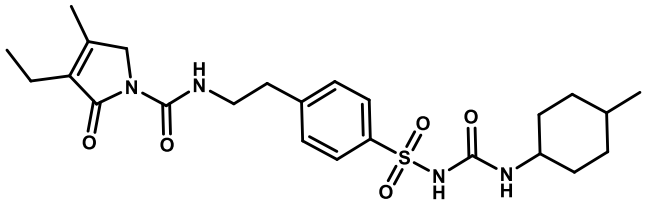


Figure 1: Formula structure of GLM

Materials and Methods

Analytical-grade chemicals and reagents were utilised throughout, and the glimepiride tablets were purchased from the marketplace. Using a 10mm matched quartz cell, Analytical Technologies Ltd.'s double beam UV-visible spectrophotometer was the instrument utilised.

Preparation of standard stock solution for Glimepiride (1000) µg/mL

Accurately weigh 0.1 g of GLM and dissolve in 100 mL of ethanol to prepare the stock solution (1000 µg/mL). Ensure proper labelling and storage in a dark bottle at room temperature.

Potassium permanganate :($3X10^{-3}$) M

Potassium permanganate ($KMnO_4$) was accurately weighed at 0.05g and dissolved in distilled water, then diluted to 100 mL in a volumetric flask. The solution was stored in a dark bottle and prepared fresh daily.

Hydrochloric acid: (0.5) M

A 0.5 M hydrochloric acid solution was prepared by measuring 10.4 mL of concentrated HCl and slowly adding it to approximately 80 mL of distilled water in a 250 mL volumetric flask with continuous stirring. The solution was then diluted to 250 mL with distilled water and stored in a labelled glass bottle at room temperature.

Interference preparation

An accurately weighed 0.1 g of the interferent was dissolved in distilled water and transferred to a 100 mL volumetric flask. The solution was then diluted to the mark with distilled water and stored in a labelled container at room temperature.

Pharmaceutical preparation

The standard solution for pharmaceutical samples was prepared using several doses of UAE-made

glybride (2-3-4) mg. Ten tablets of the drug were weighed and finely ground. A quantity of the powder, equivalent to the weight of one tablet, was accurately weighed and transferred to a volumetric flask. It was dissolved in ethanol, filtered, and then diluted to the desired concentrations.

Area Under the Absorption Curve (UAC)
UAC was calculated using UV-visible software. Optimisation included reagent volumes and temperature. Linearity, precision, accuracy, and interference studies were conducted.

Optical properties
The optical properties were computed, including molar extinction coefficients, Beer's law limit, and percentage RSD. The following regression characteristics were computed: slope, intercept, correlation coefficient, LOD, LOQ, and results of the statistical analysis of the intercept (Sa).

Validation of Methods
Construct calibration curves using at least 5 different concentrations within the linear range.
Measure each concentration in triplicate (n=3) to evaluate precision and reproducibility. Validate the method according to ICH Q2(R1) guidelines, including Linearity: Plot absorbance or UAC vs. concentration, determine the regression equation and R^2. Precision, Accuracy/Recovery: Analyse spiked samples in triplicate and calculate mean ± SD. Specificity: Confirm absence of interference from tablet excipients. LOD and LOQ: Determine based on the standard deviation of the response and slope

Results
Absorption spectra (Figure 2)

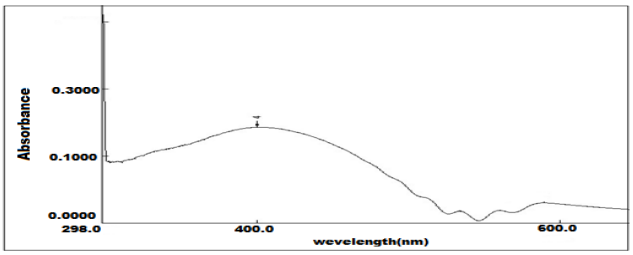


Figure 2. UV-Vis absorption spectra of glimepiride with $KMnO_4$ yellow complex at 5 µg/mL X-axis: Wavelength (nm); Y-axis: Absorbance (Abs)

Measurement of UAC from the Colour Reaction Spectrum (Figure 3 and Table 1)

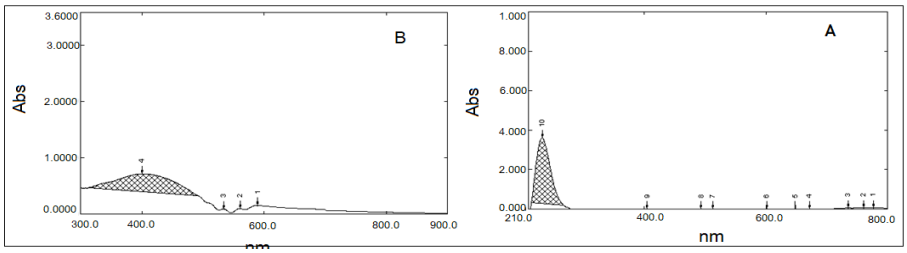


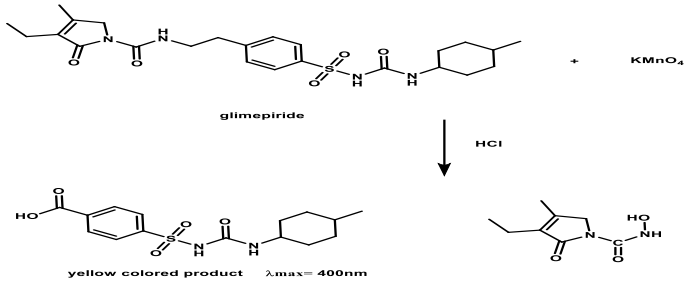
Figure 3: Comparison between the absorption spectrum of the pure drug (A) and (B) the spectrum of the product of the colourimetric reaction with the oxidising reagent using UAC measurement
Absorbance (y-axis, Abs) vs wavelength (x-axis, nm) at 5 µg/ml of glimepiride. Measurements were performed in triplicate (n=3)

Table 1: Comparison of the Area Under the Absorbance Curve (UAC) for Pure Glimepiride (A) and (B) Its Coloured Reaction Product with Potassium Permanganate

Region(A)	Start	End	Divisor	Area	Result
1	215.0	267.5	1.0000	8.7170 A (pure drug)	17.4341
Region(B)	**start**	**End**	**Divisor**	**Area**	**Result**
1	313.5	491.0	1.0000	33.6569 B (colour product)	67.3138

Integrated area under the absorption curve (Area), calculation factor (Divisor), and wavelength range (Region) are shown. The data were used to construct the calibration curve and demonstrate linearity for accurate quantification of GLM.

The proposed mechanism for the reaction of GLM with $KMnO_4$ (Scheme 1)



Scheme 1: The proposed mechanism of the reaction between KMnO4 and GLM

The stoichiometry GLM complex with $KMnO_4$ (Figure 4)

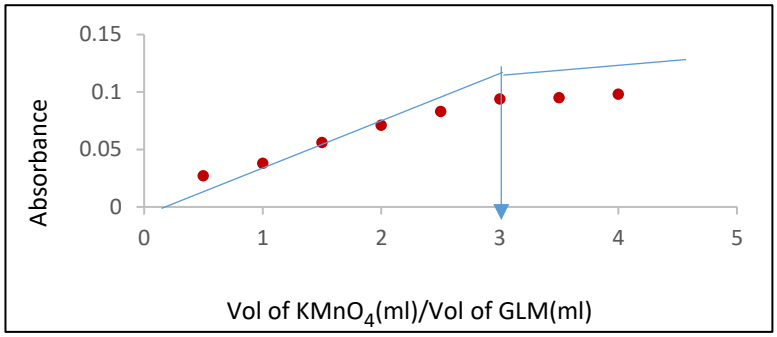


Figure 4: The mole ratio plot of GLM to $KMnO_4$, with a clear inflexion point observed at a 1:3 ratio, indicating the stoichiometry of the complex

Absorbance (y-axis, Abs) vs Volume of $KMnO_4$ /Volume of GLM (x-axis, ml)

Optimum condition (Figure 5)

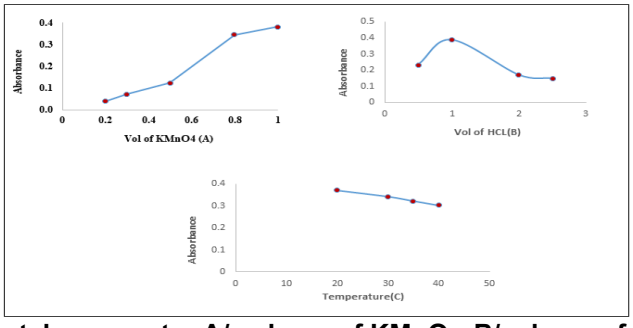


Figure 5: Chemical batch parameter A/ volume of $KMnO_4$, B/volume of HCL, C/Temperature

Absorbance (y-axis, Abs) vs Vol of $KMnO_4$, Vol of HCL, Tem (x-axis, ml, C^0)

Calibration curve (Figure 6 and Table 3)

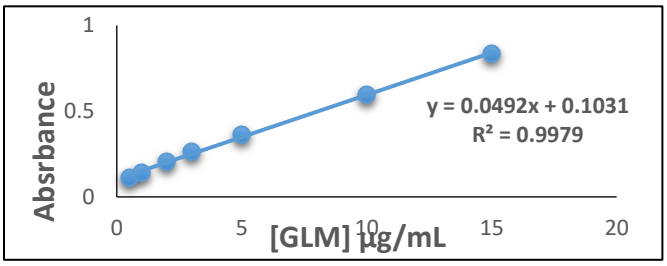


Figure 6: Glimepiride calibration curve Absorbance (y-axis, Abs) vs Con of GLM (x-axis, µg/mL)

Table 2: Accuracy and precision of the spectrophotometric method

GLM µg/mL		Error	Rec%	Erel%	RSD%
Taken	Found $\bar{x}$		n=3		n=3
3	**3.23**	**0.225**	**107.52**	**7.52**	**0.498**
5	**5.22**	**0.217**	**104.35**	**4.35**	**2.5**

Average of three determinations (Table 3)

Table 3: Analytical properties of the calibration curve

Parameters	Batch method
λmax (nm)	400
Regression equation\	y = 0.0492x + 0.1031
Linear range	0.5-15
(Rec%) n=3	101.291
(Erel%)	1.291
(RSD%) n=3	2.305
Slope	0.0492
Intercept (a); a = y - bx	0.1031
Linearity coefficient R^2	0.9979
(Sa)	0.0072
LOD n=6	0.166
LOQ n=6	0.386
(Ɛ) (L/mol.cm)	50582.92
Sandel's sensitivity	0.0091

Average of three determinations (Tables 4 and 5)

Table 4: Recovery percentage for 5 µg/mL of GLM with 1000 µg/mL of excipients present

Excipients	Conc. Found µg/mL	⁒ Recovery* n=3
Lactose	5.13	102.64
Sucrose	5.21	104.11
Sodium citrate	5.03	101.09
Glucose	5.08	100.06

*Average of three determinations.

Table 5: Determination of GLM in pharmaceutical formulations.

Sample	Labelled amount mg	Conc. taken µg/mL	Conc. found µg/mL	Recovery ٪ n=3	S.D* n=3	R.S.D*٪ n=3
Glyprid	2mg	5	4.90	98	0.002	0.714
UAE	3mg	5	5.03	100.6	0.003	0.965
	4mg	5	4.95	99	0.002	0.841

*Average of three determinations.

Discussion

The developed UV-Vis spectrophotometric method is simple, accurate, and sensitive, relying on a colourimetric oxidation reaction of GLM with $KMnO_4$ in acidic conditions. Measurement of the UAC enhanced analytical sensitivity compared to single-wavelength absorbance. Measurement of the UAC enhanced analytical sensitivity compared to single-wavelength absorbance. The sensitivity of the proposed method (LOD = 0.166 µg/mL; LOQ = 0.386 µg/mL) is superior to several previously reported UV-based methods, such as derivative spectrophotometry (LOD ≈ 0.5 µg/mL) and dye-complex assays (LOD ≈ 0.25–0.3 µg/mL). This demonstrates that the UAC approach enhances detection capability, thereby strengthening its novelty and potential for routine pharmaceutical analysis. The method demonstrates excellent linearity, precision, and accuracy, with minimal interference from tablet excipients.

Compared to previously reported techniques such as HPLC and LC-MS/MS, this method is cost-effective and suitable for routine quality control in pharmaceutical laboratories. Its reproducibility and simplicity make it a practical alternative for laboratories lacking advanced instrumentation. Limitations include reliance on freshly prepared $KMnO_4$ and controlled temperature conditions. Future studies may explore automation of UAC measurement for high-throughput screening.

The colourful product complex was produced by reacting a concentration of 5 µg/mL GLM with 1 mL 3.1×10^{-3} M of $KMnO_4$ and 1 mL 0.5 M of HCL. After examining this complex under the visible spectrum (290–600 nm), it was clear that the yellow colour complex's λ_{max} was 400 nm, as seen in Figure 2.

Measurement of UAC from the Colour Reaction Spectrum

The software supplied with the device was used to calculate the area under the curve for both the pure drug and the coloured product. Results indicated that the UAC of the reaction product has a higher absorption and a larger curve area compared to the spectrum of the pure drug. This is due to the total absorption of the product and not to the residues of the original drug or impurities, which confirms the occurrence of the reaction and the effectiveness of the method. These results indicate that the use of UAC accurately reflects the intensity of the colourimetric reaction and provides a reliable quantitative method to calculate the amount of glimepiride present in pharmaceutical samples, as shown in Figure 3 and the attached Table 1.

Investigation of the stoichiometry GLM complex with $KMnO_4$

The observed 1:3 stoichiometry from the mole ratio plot confirms the proposed complex formation between GLM and $KMnO_4$. This stoichiometric ratio is consistent with the expected reaction mechanism**.** This was accomplished by adding increased volumes of $KMnO_4$ (2×10^{-4} M) to 1 mL of GLM drug. The GLM-$KMnO_4$ complex is in a 1:3 ratio, as illustrated in Figure 4.

optimum condition

The effects of $KMnO_4$ volume were examined using 5 µg/mL GLM. Because of its high absorbance, 1 mL 3.1×10^{-3} M of $KMnO_4$ was selected for the subsequent tests. As illustrated in Figure 5, the HCL volume was measured; 1 mL of 0.5M HCL was the optimal volume. the ideal temperature was 20 degrees.

Calibration curve preparation for the batch technique

To ensure accuracy and precision, two different GLM levels were employed for these measurements, which were predicated on the optimal circumstances described in the suggested methodology. A series of 20 mL volumetric flasks containing increasing volumes of GLM standard solution was prepared. As illustrated in Figure 6, to measure GLM, a standard curve was created with a linear range of 0.5–15 µg/mL

Linearity

Different concentrations of the stock solution (100) µg/mL were used to create fresh aliquots. In a UV-visible spectrophotometer, the samples were

scanned against a reagent blank. The chosen drug exhibits linearity between 0.5 to 15 µg/mL

Precision and Accuracy
The relative standard deviation (RSD) percentage was used to compute the precision [22]. Two repeat analyses for two distinct amounts of GLM (chosen within the acquired calibration range) were performed to confirm the correctness of the proposed approach. The relative error % was then calculated and recorded in Table 2. The method's accuracy at every concentration level was demonstrated by the results.

Analytical characteristic
The λmax of glimepiride in ethanol was 400 nm. The method complies with Beer's law in the concentration range of 0.5-15 µg/mL. The optical properties calculated for Glimepiride are displayed in Table 3 and include Beer's law limits, the molar extinction coefficient (L/mol.cm), the regression equation (y), and the correlation coefficient.

Recovery percentage
Various foreign species that may be present in pharmaceutical products and the impact interaction between GLM and $KMnO_4$ have been examined under ideal experimental settings. In terms of the investigated excipients (glucose, sucrose, lactose, and sodium citrate), the results showed that there are no interferences when 1000 µg/mL is present Table 4.

Analysis of dosage forms
The previously specialised method has been developed and applied to ascertain GLM in several medicinal items. Table 5 displays the application's outcomes with reference to the recommended strategy.

Conclusion
This study presents a novel spectrophotometric method based on the area under the absorbance curve (UAC) for the quantification of glimepiride in pharmaceutical tablets, where UAC serves as a quantitative measure of the coloured product formed by the oxidation reaction with potassium permanganate in an acidic medium. The method demonstrated accuracy, sensitivity, and simplicity, making it suitable for routine laboratory applications. Some limitations, such as potential interference from tablet excipients, should be considered. Overall, UAC provides a reliable and rapid tool for glimepiride analysis.

List of Abbreviations
CE: Capillary Electrophoresis
GLM: Glimepiride
GLU: Glucose
HCl: Hydrochloric Acid
HPLC: High-Performance Liquid Chromatography
$KMnO_4$: Potassium Permanganate
LC-MS/MS: Liquid Chromatography–Tandem Mass Spectrometry
LOD: Limit of Detection
LOQ: Limit of Quantification
MIP: Molecularly Imprinted Polymer
Rec%: Recovery Percentage
R^2: Correlation Coefficient
RSD: Relative Standard Deviation
Sa: Standard Deviation of Intercept
TLC: Thin Layer Chromatography
UAC: Area Under the Absorption Curve
UPLC-MS/MS: Ultra Performance Liquid Chromatography–Tandem Mass Spectrometry
UV-Vis: Ultraviolet-Visible Spectrophotometry
RP-HPLC: Reverse Phase High-Performance Liquid Chromatography
UAE: United Arab Emirates
Erel: Relative Error
Ɛ: Molar Absorptivity / Molar Extinction Coefficient

Declarations
Ethics approval and consent
This study did not require ethical approval, as it did not involve human participants, animals, or any sensitive personal data.

Consent for publication
Not applicable

Availability of data and materials
Data available upon request

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Competing interests
None

Funding
None

Authors' contributions
WAH: Concept, methodology, manuscript drafting
ARMA: Data analysis, experiments
ASA: Literature review, experimental support
LIA: Statistical analysis, manuscript revision

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