A Research on: Methods of Development of Glimepiride and Metformin Combination by UV Spectroscopy

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ABSTRACT:
In the current era, the market is flooded with a wide range of combination dosage forms, and this number continues to increase day by day. These formulations, which consist of multiple components, have gained significant interest due to their advantages such as improved patient acceptability, increased potency, multiple actions, fewer side effects, and faster relief. Consequently, it is crucial for these formulations to meet the highest standards in terms of their quality, safety, and efficacy. Achieving this goal is only possible if various analytical techniques are available for their determination. One such technique is the use of different UV spectrophotometric methods in simultaneous multicomponent analysis. These methods rely on the recording and mathematical processing of absorption spectra. This review primarily focuses on two specific methods: the simultaneous equation method and the Q-absorbance ratio method. It provides an overview of the theories behind these methods and highlights some of their applications.

Key Words: Spectroscopic methods, Glimepiride, Simultaneous Estimation.

Introduction:
Combination drug products play a significant and time-honored role in therapeutics. When formulated in a rational manner, fixed-combination drugs can offer increased convenience, reduced cost, and sometimes improved efficacy and safety. The analysis of samples containing multiple components poses a significant challenge in modern analytical chemistry. Multicomponent analysis has emerged as a highly interesting area of study for analytical chemists in recent years, spanning various fields such as clinical chemistry, drug analysis, pollution control, and more. Various analytical techniques, including spectrophotometry, chromatography, and electrophoresis, can be utilized for multicomponent analysis. This review focuses on UV spectrophotometric methods for the simultaneous determination of drugs. Traditional UV spectral measurements are often inadequate for determining analytes of interest due to the presence of other compounds absorbing in the same spectral region in their dosage forms. Conventional extraction methods are challenging as they involve significant solvent consumption, posing risks of analyte loss or contamination, incomplete separation, and can be costly and time-consuming. UV spectrophotometric techniques are particularly useful for multicomponent analysis as they eliminate the need for separating interferents, enabling the determination of a larger number of analytes, thereby reducing analysis time and cost. Multicomponent UV spectrophotometric methods involve recording and processing absorption spectra mathematically. These methods offer several advantages, including the avoidance of prior separation techniques, ease of acquiring spectral data, speed, accuracy, simplicity, wide applicability to organic and inorganic systems, typical detection limits ranging from 10^-4 to 10^-5 M, and moderate to high selectivity. (1)

UV-SPECTROSCOPY

Principle:
Spectrophotometry is the technique that uses the absorbance of light by an analyte (the subs. To be analyzed) at a certain wavelength to determine the analyte conc. UV/VIS Spectrophotometry uses light in UV and Visible part of the Electromagnetic Spectrum. Light of this wavelength is able to effect the excitation of electrons in the atomic or molecular ground state to higher energy levels, giving rise to an absorbance at wavelengths specific to each molecule.

When a Beam of Radiation (Light) passes through a substance or a solution, some of the light may be absorbed and the remainder transmitted through the sample. The ratio of the intensity of the light entering the sample (io) the exciting the sample (I) at a particular wavelength is defined as the transmittance (T). The absorbance (A) of a sample is the negative log of transmittance. (2)
Laws of absorption

There exist two fundamental principles governing the absorption of monochromatic radiant energy by homogeneous transparent systems. These principles are known as Beer's Law and Lambert's Law.

Beer's law

The strength of a monochromatic light beam diminishes exponentially as the concentration of the absorbing substance increases linearly.

Or

The radiant energy passing through a beam of monochromatic radiation is absorbed in equal fractions by each successive increment in the number of identical absorbing molecules present in its path.

\[ I = I_0e^{-kc} \]

Where, \( I_0 \) = intensity of incident light
\( I \) = Intensity of emerged light

Lambert's law

The strength of a monochromatic radiation beam diminishes exponentially as the absorbing material's thickness increases linearly.

Or

When light passes through a transparent medium, the intensity of the transmitted light decreases at a rate that is directly proportional to the intensity of the incident light and the thickness of the medium.

\[-\frac{dI}{dt} \propto I\]

The empirical expression known as Beer and Lambert's Law can be derived from the aforementioned laws.

\[ A = \varepsilon ct \]

Where, \( A \) = Absorbance or optical density or extinction co-efficient
\( \varepsilon \) = Molecular extinction co-efficient
\( c \) = Concentration of drug
\( t \) = Path length (3)

According to no. of the Component used, there are two analytical methods of UV Visible Spectroscopy


1. Single Component Analysis Method:

It Consist of two methods:

- Direct Analysis - Direct analysis involves the measurement of compounds that contain conjugated double bonds or aromatic rings, as well as various inorganic species, as they have the ability to absorb light in the UV-visible region. In this method, the substance of interest is dissolved in an appropriate solvent and then diluted to the desired concentration. The absorbance of the solution is then measured to determine the concentration of the compound or species being analyzed.

- Indirect Analysis - The chemical agent is utilized to convert the analyte, as the analyte exhibits weak absorption in the UV region. This conversion helps in preventing interference from irrelevant compounds by transforming the analyte into a derivative that absorbs in the visible region, where the absorption of irrelevant compounds is minimal (5)
2. Multi Component Analysis Methods:

(A) Simultaneous equation method (Vierordt’s method)

In cases where a sample includes two absorbing drugs (x and y) with absorption occurring at the λ max of the other, it is feasible to identify both drugs using the simultaneous equation technique (Vierordt’s method) under specific conditions.

The information required is

1. The absorptivities of x at wavelengths λ1 and λ2 are denoted as ax1 and ax2, while for y they are represented as ay1 and ay2 respectively.
2. The absorbance values of the diluted samples at λ1 and λ2 are indicated as A1 and A2 correspondingly.

Cx and Cy represent the concentrations of x and y, respectively, in the diluted samples. Two equations are formulated by considering the fact that at λ1, the absorbance of the mixture is equal to the combined absorbance of x and y.

\[ A_1 = a_1 c_x + a_1 c_y \] …………. (1)
\[ A_2 = a_2 c_x + a_2 c_y \] …………. (2)

For measurements in 1 cm cells, b =1cm. Rearrange Eq. (2)

\[ c_y = (A_2 - a_2 c_x)/a_2 \] ……………… (3)

Substituting for Cy in eq. (1) and rearranging gives

\[ c_x = (A_1 - a_1 c_x)/a_1 \] ……………… (4)

\[ c_y = (A_1 a_2 - A_2 a_1)/a_1 a_2 \] ……………… (5)

Suggested guidelines have been proposed to achieve maximum precision in determining the concentrations of components in a mixture, based on absorbance ratios. These guidelines specify that the ratios (A2/A1)/ax2/ax1) and (ay2/ay1)/(A2/A1) should fall outside the range of 0.1-2.0 for the accurate determination of x and y, respectively. These criteria are met only when the λ max values of the two components are significantly different and when there is no chemical interaction between the components, thus invalidating the initial assumption that the total absorbance is the sum of the individual absorbances. The simultaneous equation method has been developed to enable the simultaneous determination of multiple mixtures, such as atenolol and indapamide, as well as dextubiprofen and paracetamol. (1)

(B) Absorbance Ratio Method (Q - ANALYSIS)

The absorbance ratio method is a variant of the simultaneous equation’s procedure. Its fundamental principle is that if any absorbing species follows Beer’s law at all wavelengths, the absorbance ratio at any two wavelengths remains constant regardless of concentration or pathlength. In this method, the absorbance of a standard solution for each component is measured at the isobestic point (λ1) and the wavelength of maximum absorption for one of the two components (λ2). The absorptivity coefficients of the pure component at both wavelengths are then calculated. Similarly, the absorbance of the sample is also determined at λ1 and λ2.

From the following sets of equations, the concentration of each component (CX & CY) in sample can be calculated 10:

\[ C_x = (Q_x - Q_y) A_M & C_Y = (Q_M - Q_Y) A_M / (Q_x - Q_y) a_1 x_1 \]
\[ Q_M = (A_{sample \\ at \\ λ2})/(A_{sample \\ at \\ λ1}) \]
\[ Q_x = (A_{pure \\ component \\ X \\ at \\ λ2})/(A_{pure \\ component \\ Y \\ at \\ λ1}) \]
\[ Q_y = (A_{pure \\ component \\ Y \\ at \\ λ2})/(A_{pure \\ component \\ Y \\ at \\ λ1}) \]
\[ a_1 x_1 = A_{sample \\ at \\ λ1}/A_{sample \\ at \\ isobestic \\ (λ1) \\ wavelength} \]
\[ a_1 y_1 = A_{sample \\ at \\ λ1}/A_{sample \\ at \\ isobestic \\ (λ1) \\ wavelength} \]

(C) Derivative Spectroscopic Method

Derivative spectrophotometry is a technique that aims to enhance the accuracy of spectral analysis by converting a regular spectrum (also known as a fundamental, zeroth order, or D spectrum) into its first, second, or higher derivative spectrum. This is achieved by differentiating the absorbance of a sample with respect to the wavelength λ.

\[ [A] = f(λ), \text{ zero order} \]
\[ [dA/dλ] = f(λ), \text{ first order} \]
\[ [d^2A/dλ^2] = f(λ), \text{ second order} \]

The inflection point in the absorbance band, characterized by strong positive and negative bands with maximum and minimum at the same wavelength, plays a crucial role in governing the odd (first and third) derivative spectrum. On the other hand, the even (second and fourth) derivative spectrum is governed by the strong positive and negative band with minimum or maximum at the same wavelength as the λ max of the absorbance band.

Number of bands = Derivative order + 1

The magnitude of the signal (D) increases in direct correlation with the concentration of the substance being analyzed, assuming that the D° spectrum follows Beer’s law.

In first-order derivative spectroscopy, the zero-crossing point is identified for both drugs, with wavelengths chosen so that when one drug reaches its zero-crossing point, the other drug exhibits significant absorbance. (6)

(D) Difference Spectrophotometry

Spectrophotometric analysis of samples with absorbing interferents can be enhanced significantly by utilizing difference spectrophotometry. This method involves measuring the absorbance difference (ΔA) between two equimolar solutions of the analyte in distinct chemical forms with varying
spectral characteristics. The application of difference spectrophotometry in substance assays alongside other absorbing substances requires specific criteria to be met.

1. The addition of one or more reagents can introduce reproducible modifications in the spectrum of the analyte.
2. The reagent does not modify the absorbance of the interfering substances. The adjustment of the pH using aqueous solutions of acids, alkalis, or buffers is the simplest and most frequently used method to modify the spectral properties of the analyte. (1)

### Glimepiride:

Glimepiride, classified as a sulfonylurea, is a medication commonly used to treat type 2 diabetes by helping to regulate blood sugar levels. Glimepiride is considered a secondary choice when compared to metformin, primarily because metformin has a well-documented track record of being safe and effective. To maximize its benefits, glimepiride is typically prescribed alongside lifestyle changes, including dietary adjustments and regular physical activity. This medication is administered orally and reaches its maximum effectiveness within three hours, providing a sustained effect for approximately 24 hours. (7,8).

Typical adverse reactions consist of headache, nausea, and vertigo. Severe adverse effects could involve hypoglycemia. The utilization of this medication is discouraged during pregnancy and lactation. Its primary mechanism of action involves enhancing the secretion of insulin from the pancreas. This drug is categorized as a second-generation sulfonylurea. (9,10).

#### Structure of glimepiride:

![Glimepiride Structure](image)

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trade names:</td>
<td>Amaryl, others</td>
</tr>
<tr>
<td>Routes of administration:</td>
<td>by mouth</td>
</tr>
<tr>
<td>Bioavailability</td>
<td>100%</td>
</tr>
<tr>
<td>Protein binding</td>
<td>&gt;99.5%</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Complete Liver (1st stage through CYP2C9)</td>
</tr>
<tr>
<td>Onset of action</td>
<td>2–3 hours</td>
</tr>
<tr>
<td>Elimination half-life</td>
<td>5–8 hours</td>
</tr>
<tr>
<td>Duration of action</td>
<td>24 hours</td>
</tr>
<tr>
<td>Excretion</td>
<td>Urine (~60%), feces (~40%)</td>
</tr>
<tr>
<td>Formula</td>
<td>C24H34N4O5S</td>
</tr>
<tr>
<td>Molar mass</td>
<td>490.62 g·mol⁻¹</td>
</tr>
<tr>
<td>Melting point</td>
<td>207 °C (405 °F)</td>
</tr>
</tbody>
</table>

**IUPAC Name:**


**Medical uses:**

Glimepiride is prescribed for the treatment of type 2 diabetes, as it works by enhancing insulin secretion from the pancreas. Nevertheless, it is essential to have sufficient insulin production in order to effectively manage the condition. This medication is not suitable for type 1 diabetes, as individuals with type 1 diabetes have impaired insulin production in the pancreas. (11).

**Mechanism of action:**

Like all sulfonylureas, glimepiride acts as an insulin secretagogue. It lowers blood sugar by stimulating the release of insulin by pancreatic beta cells and by inducing increased activity of intracellular insulin receptors.

Not all secondary sulfonylureas have the same risk of hypoglycemia. Glibenclamide (glyburide) is associated with an incidence of hypoglycemia of up to 20–30%, compared to as low as 2% to 4% with glimepiride. Glibenclamide also interferes with the normal homeostatic suppression of insulin secretion in reaction to hypoglycemia, whereas glimepiride does not. Also, glibenclamide diminishes glucagon secretion in reaction to hypoglycemia, whereas glimepiride does not. (12,13).
Pharmacokinetics:
Gastrointestinal absorption is complete, with no interference from meals. Significant absorption can occur within one hour, and distribution is throughout the body, 99.5% bound to plasma protein. Metabolism is by oxidative biotransformation; it is hepatic and complete. First, the medication is metabolized to M1 metabolite by CYP2C9. M1 possesses about 1⁄3 of pharmacological activity of glimepiride, yet it is unknown if this results in clinically meaningful effect on blood glucose. M1 is further metabolized to M2 metabolite by cytosolic enzymes. M2 is pharmacologically inactive. Excretion in the urine is about 65%, and the remainder is excreted in the feces.

Adverse effects:
Glimepiride is prescribed for the treatment of type 2 diabetes, as it works by enhancing insulin secretion from the pancreas. Nevertheless, it is essential to have sufficient insulin production in order to effectively manage the condition. This medication is not suitable for type 1 diabetes, as individuals with type 1 diabetes have impaired insulin production in the pancreas. (14)

Metformin:
Metformin, marketed as Glucophage and other brands, is the primary initial medication for managing type 2 diabetes, especially in individuals who are overweight. Additionally, it is utilized in the treatment of polycystic ovary syndrome. In certain cases, it is prescribed off-label to reduce the likelihood of metabolic syndrome in individuals taking antipsychotics. Metformin is administered orally and does not contribute to weight gain. Metformin functions as a biguanide anti-hyperglycemic agent by reducing glucose production in the liver, enhancing the insulin sensitivity of bodily tissues, and boosting GDF15 secretion to lower appetite and caloric consumption. (15,16,17).

Structure of Metformin:

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trade names</td>
<td>Fortamet, Glucophage, Glumetza</td>
</tr>
<tr>
<td>Other names</td>
<td>N, N-dimethyl biguanide.</td>
</tr>
<tr>
<td>Route of Administration</td>
<td>by mouth</td>
</tr>
<tr>
<td>Bioavailability</td>
<td>50–60%</td>
</tr>
<tr>
<td>Protein binding</td>
<td>Minimal</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Not by liver</td>
</tr>
<tr>
<td>Elimination half-life</td>
<td>4–8.7 hours</td>
</tr>
<tr>
<td>Excretion</td>
<td>Urine (90%)</td>
</tr>
<tr>
<td>IUPAC Name</td>
<td>N, N-Dimethylimidodicarbonimidic diamide</td>
</tr>
<tr>
<td>Formula</td>
<td>C4H11N5</td>
</tr>
<tr>
<td>Molar mass</td>
<td>129.167 g·mol⁻¹</td>
</tr>
<tr>
<td>Density</td>
<td>1.3±0.1[11] g/cm³</td>
</tr>
</tbody>
</table>

Medical uses:
Metformin is prescribed to reduce blood glucose levels in individuals diagnosed with type 2 diabetes. Additionally, it serves as a secondary treatment option for infertility in individuals with polycystic ovary syndrome. (18)

Mechanism of action:
Metformin's mechanisms of action are unique from other classes of oral antihyperglycemic drugs. Metformin decreases blood glucose levels by decreasing hepatic glucose production (also called gluconeogenesis), decreasing the intestinal absorption of glucose, and increasing insulin sensitivity by increasing peripheral glucose uptake and utilization. It is well established that metformin inhibits mitochondrial complex I activity, and it has since been generally postulated that its potent antidiabetic effects occur through this mechanism. The above processes lead to a decrease in blood glucose, managing type II diabetes and exerting positive effects on glycemic control. (21,22)

After ingestion, the organic cation transporter-1 (OCT1) is responsible for the uptake of metformin into hepatocytes (liver cells). As this drug is positively charged, it accumulates in cells and in the mitochondria because of the membrane potentials across the plasma membrane as well as the...
mitochondrial inner membrane. Metformin inhibits mitochondrial complex I, preventing the production of mitochondrial ATP leading to increased cytoplasmic ADP: ATP and AMP:ATP ratios. These changes activate AMP-activated protein kinase (AMPK), an enzyme that plays an important role in the regulation of glucose metabolism. (23) Aside from this mechanism, AMPK can be activated by a lysosomal mechanism involving other activators. Following this process, increases in AMP:ATP ratio also inhibit fructose-1,6-bisphosphatase enzyme, resulting in the inhibition of gluconeogenesis, while also inhibiting adenylyl cyclase and decreasing the production of cyclic adenosine monophosphate (cAMP), a derivative of ATP used for cell signaling. Activated AMPK phosphorylates two isoforms of acetyl-CoA carboxylase enzyme, thereby inhibiting fat synthesis and leading to fat oxidation, reducing hepatic lipid stores and increasing liver sensitivity to insulin.

In the intestines, metformin increases anaerobic glucose metabolism in enterocytes (intestinal cells), leading to reduced net glucose uptake and increased delivery of lactate to the liver. Recent studies have also implicated the gut as a primary site of action of metformin and suggest that the liver may not be as important for metformin action in patients with type 2 diabetes. Some of the ways metformin may play a role on the intestines is by promoting the metabolism of glucose by increasing glucagon-like peptide 1 (GLP-1) as well as increasing gut utilization of glucose. (21,24)

Pharmacokinetics:
Metformin has an oral bioavailability of 50–60% under fasting conditions, and is absorbed slowly. Peak plasma concentrations (Cmax) are reached within 1–3 hours of taking immediate-release metformin and 4–8 hours with extended-release formulations. The plasma protein binding of metformin is negligible, as reflected by its very high apparent volume of distribution (300–1000 L after a single dose). Steady state is usually reached in 1–2 days. (19)

Adverse effects:
Metformin is known to cause gastrointestinal irritation, which includes symptoms like diarrhea, cramps, nausea, vomiting, and increased flatulence. Compared to other antidiabetic medications, metformin is more likely to cause these gastrointestinal adverse effects. However, the most severe potential adverse effect of metformin is lactic acidosis, although this complication is rare. Lactic acidosis appears to be linked to impaired liver or kidney function. It’s important to note that metformin is not recommended for individuals with severe kidney disease, but it may still be prescribed at lower doses for those with kidney problems. (20)

Marketed preparation of Glimepiride and Metformin:

1. Glycomet - GP:

2. Gluconorm-G1:

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3. Gemer:

![Gemer Image]

4. Glimestar-M

![Glimestar-M Image]

5. Azulix MF

![Azulix MF Image]
6. Glimisave M

Materials:

Instrumentation:
The experiment employed a Varian Cary 100 double beam UV-Visible spectrophotometer, equipped with 10mm matched quartz cells. To measure the weight accurately, a Shimadzu AUW-220D electronic balance was utilized.

Fig: Double beam UV-Visible spectrophotometer (Varian Cary 100)

Fig: Electronic balance (Model Shimadzu AUW-220D)
Methods

Method A. Simultaneous Estimation Method

The determination of λ max for Glimepiride and Metformin involved scanning the drug solution in a UV Spectrophotometer within the wavelength range of 200 - 400 nm, with a band width of 0.5 and a scan speed of 600 nm/min. The λ max values obtained were 258.02 nm for Glimepiride and Metformin, respectively. In order to construct Beer’s plot for both drugs, stock solutions of 1000 µg/ml were prepared in methanol, and working standard dilutions were made using these stock solutions. Beer’s plot was also constructed for the drug mixture at various concentration levels. It was observed that both Glimepiride and Metformin exhibited linearity both individually and in mixture within the concentration ranges of 2-10 µg/ml and 6-30 µg/ml, respectively.

Method B. Absorption Corrected Method

The determination of λ max for Glimepiride and Metformin involved scanning the drug solution in a UV Spectrophotometer within the wavelength range of 200 - 400 nm, with a band width of 0.5 and a scan speed of 600 nm/min. The λ max values obtained were 258.02 nm and 299.85 nm for Glimepiride and Metformin, respectively. In order to construct Beer’s plot for both drugs, stock solutions of 1000 µg/ml were prepared in methanol, and working standard dilutions were made using these stock solutions. Beer’s plot was also constructed for the drug mixture at various concentration levels. It was observed that both Glimepiride and Metformin exhibited linearity both individually and in mixture within the concentration ranges of 2-10 µg/ml and 6-30 µg/ml, respectively. (25)

Preparations

Preparation of Standard Stock Solutions and Calibration Curve

Pure drug stock solutions containing 1000 µg/mL of Glimepiride and Metformin were prepared individually in methanol. These standard stock solutions were then diluted with methanol to obtain working standard solutions of the analytes. The concentration range for Glimepiride was 2-10 µg/ml, while for Metformin (Metformin) it was 6-30 µg/ml. The working standard solutions were scanned in the wavelength range of 200-400nm.

To prepare calibration curves for both drugs, the first derivative amplitudes of the spectra were determined using the aforementioned procedures. It was observed that Beer’s law was followed within the concentration range of 2-10 µg/mL for Glimepiride and 6-30 µg/mL for Metformin, using both methods.

Preparation of Sample Solution and Formulation Analysis

Twenty tablets were precisely weighed, and an amount of tablet powder equivalent to 10 mg of Glimepiride (Metformin 30mg) was accurately weighed and dissolved in 30 mL of methanol using ultra-sonication for 10 minutes. The resulting solution was then filtered through Whatman paper No. 41 into a 100 mL volumetric flask, and the volume was adjusted to the mark with methanol. Subsequently, the solution was appropriately diluted with methanol to achieve concentrations of 10 µg/mL for Glimepiride and 30 µg/mL for Metformin. The percentage of labelled claim and the standard deviation (S.D) were determined, and the findings are detailed in Table 1.

Recovery studies

The proposed methods’ accuracy was assessed through recovery studies, which involved adding a standard drug solution to a reanalysed sample solution at three distinct concentration levels (50%, 100%, and 150%) within the linearity range for both drugs. The chosen concentration level for spiking the drugs' standard solution into the sample solution was 10 µg/ml of Glimepiride and 30 µg/ml of Metformin for both methods.

Validations

Precision

The repeatability of the method was assessed by performing the assay procedure six times. To evaluate the intra-day precision, the method was repeated five times within a single day, and the average % RSD was calculated. Similarly, the method was repeated on five different days to determine the inter-day precision, and the average % RSD was determined (Table 1). Additionally, the precision of the analyst was determined by having another analyst in the laboratory repeat the study.

Specificity

Specificity is a method utilized to quantitatively identify the analyte in the presence of other components that could potentially exist in the sample matrix. Excipients commonly found in tablet formulations were added to a pre-determined number of drugs, followed by the measurement of absorbance and subsequent calculations to ascertain the drug quantity.

Robustness

The proposed methods were evaluated for their robustness through variations in parameters like wavelength range and slit width. It was observed that alterations in these variables did not have a significant impact on the absorbance of the drugs, suggesting that the methods can be deemed robust.
Limit of Quantification (LOQ) and Limit of Detection (LOD)
The limits of detection (LOD) and quantification (LOQ) for Glimepiride and Metformin were established through the suggested techniques by utilizing calibration standards. The LOD and LOQ values were computed as 3.3 times the standard deviation (σ) divided by the slope (S) and 10 times σ divided by S, respectively. The outcomes are presented in Table 1.

Results:

![Fig. No. 2: Simple Overlay Spectra of Glimepiride and Metformin (Absorbance corrected)](image_url)

![Fig. No. 3: Simple spectra of Metformin.](image_url)
Fig. No. 2 Simple Overlay Spectra of Glimepiride and Metformin (Simultaneous Estimation Method)

### Table 1: Optical characteristics of the proposed methods and result of formulation analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Glimepiride</th>
<th>Metformin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Method A</td>
<td>Method B</td>
</tr>
<tr>
<td>wavelength (nm)</td>
<td>258.02</td>
<td>301.87</td>
</tr>
<tr>
<td>Beer’s law limit (μg/mL)</td>
<td>2-10</td>
<td>2-10</td>
</tr>
<tr>
<td>Regression Equation* Slope (m)</td>
<td>0.00533</td>
<td>0.00516</td>
</tr>
<tr>
<td></td>
<td>0.009365</td>
<td>-0.0066</td>
</tr>
<tr>
<td>Regression Equation* Intercept (c)</td>
<td>-0.011012</td>
<td>-0.00379</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.996</td>
<td>0.999</td>
</tr>
<tr>
<td>Formulation Analysis (% Assay, %RSD), n=6</td>
<td>99.12, 0.56</td>
<td>100.2, 0.98</td>
</tr>
<tr>
<td>LOQ (μg/mL)</td>
<td>0.5234</td>
<td>0.6735</td>
</tr>
<tr>
<td>LOQ (μg/mL)</td>
<td>2.1215</td>
<td>1.962</td>
</tr>
<tr>
<td>Ruggedness Analyst I</td>
<td>1.08</td>
<td>0.95</td>
</tr>
<tr>
<td>Ruggedness Analyst II</td>
<td>0.87</td>
<td>1.14</td>
</tr>
<tr>
<td>RSD = Relative Standard Deviation, Y* = mX + c, where Y is the absorbance and X the concentration (μg/mL)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Accuracy result for Glimepiride & Metformin

<table>
<thead>
<tr>
<th>Recovery Level</th>
<th>Analytename</th>
<th>Amount/Spiked (μg/mL)</th>
<th>% Mean Recovery, % RSD, n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>Glimepiride</td>
<td>10</td>
<td>98.12, 0.65</td>
</tr>
<tr>
<td></td>
<td>Metformin</td>
<td>37</td>
<td>99.53, 0.76</td>
</tr>
<tr>
<td></td>
<td>Glimepiride</td>
<td>20</td>
<td>99.50, 1.02</td>
</tr>
</tbody>
</table>
### Conclusion:

The validated spectrophotometric methods employed here proved to be simple, economical, precise and accurate. Thus, it can be used as IPQC test and for routine simultaneous determination of Glimepiride and Metformin in tablet dosage form.

### REFERENCES:

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