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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN AND GLICLAZIDE IN BULK DOSAGE FORM

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ABSTRACT

The goal of the current work is to create a straightforward, quick, affordable and UV-visible spectrophotometric derivative approach for the simultaneous measurement of gliclazide and metformin hydrochloride in pharmaceutical dosage forms and bulk. By using a solvent solution made up of distilled water and sodium hydroxide (NaOH), the technique improves drug solubility while using fewer hazardous organic solvents that are frequently used in traditional analytical techniques. To find the maximum absorption wavelengths of both medications, standard stock solutions were made and scanned over the UV spectrum. The highest absorbance of gliclazide and metformin hydrochloride was observed at about 229 and 233 nm, respectively. The simultaneous equation method was used to quantitatively estimate the medicines and calibration curves were built within appropriate concentration ranges. For criteria including linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ), the developed analytical technique was validated in accordance with standard guidelines. The validation findings showed great accuracy, adequate precision and decent linearity. As a result, the suggested spectrophotometric technique can be successfully used for regular quality control analysis of gliclazide and metformin hydrochloride in pharmaceutical formulations.

KEYWORDS: Metformin Hydrochloride, Gliclazide, UV-Visible Spectrophotometry,

Simultaneous Estimation, ICH Guidelines, Quality Control.

1. INTRODUCTION

1.1 Introduction to Disease

Diabetes: Diabetes disrupts the body's capacity to make or utilize insulin, which can have an impact on every part of the body. When your body converts the food you eat into energy (sugar or glucose), insulin aids in the transportation of energy to the cell.

1) Diabetes type I

Five to ten percent of people with diabetes are diagnosed with type I diabetes, which is brought on by a complete lack of insulin secretion. The immune system's death of the pancreatic β cells that produce insulin is the cause of this obvious lack of insulin production.

2) Diabetes type II

A common disorder known as type 2 diabetes occurs when the body either produces insufficient amounts of insulin or is unable to use it effectively. Roughly 90% of diabetes cases globally are caused by it. It can be controlled by eating a balanced diet, exercising frequently, keeping an eye on blood sugar levels and maintaining a healthy weight. But because it is a progressive illness, it may get worse over time and frequently calls for insulin or medicine. This illness is more likely to develop in those who are overweight or have considerable abdominal fat.

3) Gestational Diabetes

A form of diabetes known as gestational diabetes develops during pregnancy when the body is unable to create enough insulin, which raises blood sugar levels. It is typically treatable with a nutritious diet and consistent exercise and it is identified during pregnancy.

Nonetheless, 10–20% of women could require medication to manage their blood sugar levels. It can raise the chance of difficulties during labour if improperly managed.

Oral hypoglycemic agents

1) Sulfonylureas Class: e.g. Gliclazide, Glipizide.
2) Biguanides Class: e.g., Metformin.

Gliclazide and metformin hydrochloride are two common oral antidiabetic medications used to treat type 2 diabetes. While gliclazide increases insulin release from pancreatic β-cells, met

formin increases insulin sensitivity and reduces hepatic glucose synthesis. These medications are frequently combined in pharmaceutical formulations to improve glycemic control.

1.2 Introduction to spectroscopy

The measurement and interpretation of Electromagnetic Radiation (EMR) absorbed or released when a sample's molecules, atoms, or ions transition from one energy state to another is known as spectroscopy.

Principle

The theory behind principle of UV-Visible Spectroscopy is that chemical substances can absorb visible or ultraviolet light and produce unique spectra in the process. The interaction of light and matter is the foundation of spectroscopy. The fundamental idea behind absorbance spectroscopy is the Beer-Lambert Law. The following formulas are applied to a single wavelength: A = absorbance (usually represented as arb, unit less). units or arbitrary units) a = molar absorptivity (M -1 cm-1), b = cuvette or sample container route length (often 1 cm) and c = solution concentration (M).

Where, A: Absorbance a: Absorptivity b: path length c: Concentration C: A /a b.

1.3 Introduction to UV–Visible Spectrophotometer

- **Instrumentation of UV–Visible Spectrophotometer (LABMAN LMSPUV-1900):**

Over the past 40 years, ultraviolet and visible spectrometers have become the most crucial analytical tool in contemporary laboratories. UV-visible spectrometry is utilized because of its ease of use, adaptability, speed, accuracy and affordability, while other methods may also be applied. Our senses of sight and radiant heat serve as frequent reminders that radiation is a type of energy [11].A double beam UV-visible spectrophotometer (LABMAN, Model LMSPUV-1900) was used to perform the UV-visible spectrophotometric analysis. The device operates in the 190–1100 nm wavelength range, allowing for analysis in both the visible and ultraviolet spectrums.

Figure No 01: UV Spectrophotometer.

- **Principle of Instrument:** The device operates on the basis of Beer-Lambert's law, which states that molecules absorb ultraviolet or visible light. The concentration of the analyte is directly correlated with the solution's absorbance. Components of UV Spectrophotometer:- Light sources**,** Monochromator ,Sample cell, Detector**,** Display and Data System**,** Control System.

2. Analytical Validation Parameters as Per ICH Guidelines

Validation is the process of obtaining experimental evidence of an analytical technique's ability to provide results with the required precision and accuracy. To evaluate the quality qualities of pharmaceuticals, the analytical techniques used in their manufacturing must be validated. It is not essential to evaluate the suitability of the methods outlined and recorded in the State Pharmacopoeia as long as the analyses are conducted precisely in accordance with the text of each individual item. Therefore, a crucial part of the steps a laboratory should take to enable the production of trustworthy analytical data is method validation. The Association of Official Analytical Chemists, International Conference on Harmonization, Pharmacopoeias and Eurachem papers provide the majority of the protocols and guidelines on technique validation and uncertainty. The validation of the analytical method should show that it is appropriate for its intended use. The method's scope, performance characteristics and acceptability constraints are all part of the strategy that the validation adheres to: Limits of detection and quantification, accuracy, precision, linearity, robustness and ruggedness are among the parameters that are typically looked at throughout the validation process.

1. Accuracy

The degree to which the measured value closely resembles the true value is known as measurement accuracy. Accuracy can be assessed in three ways.

2. Precision

The degree of agreement between individual test results when a process is applied repeatedly to several samplings of a homogenous sample is known as precision.

Types of precision

1. Repeatability (Intra-day Precision) - Analysis performed on same day.
2. Intermediate Precision (Inter-day Precision)- Analysis performed on different day.

3. Linearity

The degree to which a calibration plot of response vs concentration resembles a straight line is a measure of a method's linearity. Single measurements at various analyte concentrations are used to carry it out. After that, a linear least square regression is used to process the data. The required linearity information is provided by the slope, intercept and coefficient of correlation. For the majority of approaches, a correlation coefficient greater than 0.999 is acceptable.

4. Limit of Detection (LOD)

The lowest concentration of analyte in a sample that can be detected but not always quantified under specified experimental circumstances is known as the detection limit of an analytical process. The expression for the detection limit is

DL = 3.3 σ / S.

where, S is the calibration curve's slope.

σ is the response's standard deviation.

5. Limit of Quantification (LOQ)

The lowest amount of analyte in a sample that can be quantitatively identified with appropriate precision and accuracy is known as the quantitation limit of a particular analytical process. The quantitation limit can be written as DL = 10 σ / S

Where, σ is the response's standard deviation. The calibration curve σ slope S

6. Ruggedness

The reproducibility of results when the method is used in real-world scenarios is known as ruggedness.

7. Robustness

ICH defines robustness as a measure of an analytical procedure's ability to withstand slight but intentional changes in technique parameters.

Characteristics to be validated

Table No.01

Characteristics	Acceptance Criteria
Accuracy	Recovery 98 – 102% (individually)
Precision	RSD < 2%
Detection Limit	S/N > 2 or 3
Quantitation Limit	S/N > 10
Linearity	Correlation coefficient r2 > 0.999

3. OBJECTIVES

1) To create a straightforward and affordable UV-visible spectrophotometric technique for the simultaneous measurement of metformin hydrochloride and gliclazide in a combination dose form.
2) To choose appropriate wavelengths so that both medications can be accurately analyzed without first being separated.
3) To determine the linearity of metformin hydrochloride and gliclazide across a suitable concentration range.
4) To use the established approach for regular pharmaceutical tablet quality control analysis.
5) To verify the suggested method's accuracy, precision and repeatability in accordance with accepted analytical standards.

4. MATERIALS & METHODS

Instrument

A UV–Visible Spectrophotometer, **Model -** LABMAN LMSPUV-1900, Double beam Spectrophotometer.

Materials

Gift samples of Metformin and Gliclazide were procured from Micro Labs Limited CTS No.73, Saki Estate, Off Chandivali Road, Kurla(W), Mumbai400072,Maharashtra,India. Respectively.

Solvent used: 0.1 N NaOH

Figure No.02

Figure No.03

Method: Simultaneous Equation Method

Standard stock solutions of Gliclazide and Metformin API were prepared separately by dissolving accurately weighed drug powder in NaOH solution. From the stock solutions, suitable working standard solutions were prepared by proper dilution using the same NaOH solvent. These solutions were scanned in the UV-visible region and two wavelengths were selected: 226 nm for Gliclazide and 235 nm for Metformin These wavelengths were chosen because they showed the maximum absorbance (λmax) of both drugs. A series of standard dilutions were prepared: Gliclazide: 3–15µg/mL and Metformin: 3–15 µg/mL &Each dilution's absorbance was measured at 226 and 235 nm. Both medications' calibration curves and absorptivity coefficients were computed using these measurements. A mixed API solution including metformin and gliclazide was made in NaOH solvent for sample analysis. After the solution was fully dissolved and put into a 100 mL volumetric flask, the volume was adjusted with NaOH. If necessary, the final solution was filtered and additional dilutions were made to achieve the necessary concentration range. The sample solution's absorbance was measured at: o 226 nm o 235 nm. The concentration of both drugs was then calculated using the following simultaneous equations:A1= ax1 b cx + ay1 b cy and A2= ax2 b cx + ay2 b cy

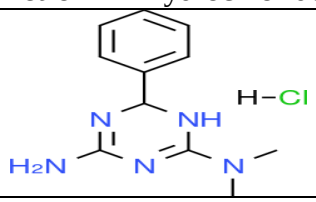
Where; Cy = concentration of Gliclazide Cx= concentration of Metformin A_1 = absorbance at 226 nm

A_2 = absorbance at 235nm

5. Drug Profile

5.1 Drug Profile for Metformin HCL

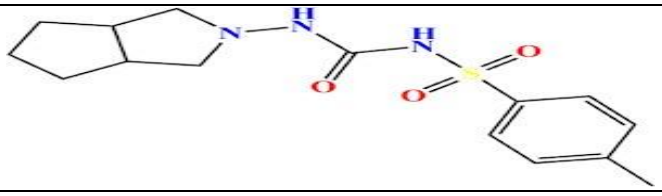
Table No. 02

Name of the Drug	Metformin Hydrochloride
Chemical Structure	
Molecular Formula	C4H11N5.HCL
Molecular Weight	165.6g/mol
IUPAC Name	3-(diamino methylidene)-1,1 dimethyl guanidine; hydrochloride
CAS No.	1115-70-4
Category	Antidiabetic agent
Solubility	Sparingly soluble in methanol; slightly soluble in alkali, soluble in water.
Wavelength	235nm

Physico-chemical properties	
Appearance	Solid
Colour	White
Characteristics	A White, crystalline powder; hygroscopic.
Melting point	223-226 °C
Pka	12.4
Mechanism Action	Metformin inhibits mitochondrial complex I activity and it has since been generally postulated that potent Antidiabetic effects.
Therapeutic use	Metformin is indicated as an adjunct to diet and exercise to increase glycaemic control in adults.

5.2 Drug Profile for Gliclazide

6. Table No. 03.

Name of the Drug	Gliclazide
Chemical Structure	
Molecular Formula	$C_{15}H_{21}N_3O_3S$
Molecular Weight	323.41g/mol
IUPAC Name	1-[(4-methylbenzene)sulfonyl]-3 {octahydrocyclopenta[c]pyrrol-2-yl}urea
CAS No.	21187-98-4
Category	Antidiabetic agent
Solubility	Partially insoluble in water, freely soluble in methylene chloride, sparingly soluble in acetone, slightly soluble in ethanol(96%).
Wavelength	226 nm
Physico-chemical properties	
Appearance	Solid
Colour	White
Characteristics	White crystalline powder, odourless
Melting point	181 °C
Pka	14.13
Mechanism Action	Gliclazide binds to sulfonylurea receptors on pancreatic β-cells and blocks ATP-sensitive K+ channels. This reduces K+ efflux, causing cell depolarization which opens voltage-gated Ca^{2+} channels. The resulting Ca^{2+} influx triggers insulin granule exocytosis, increasing insulin release to lower blood glucose.
Therapeutic use	Used in the treatment of Type 2 Diabetes Mellitus to control blood glucose levels.

7. SpectralAnalysis

Metformin Hydrochloride **Gliclazide**

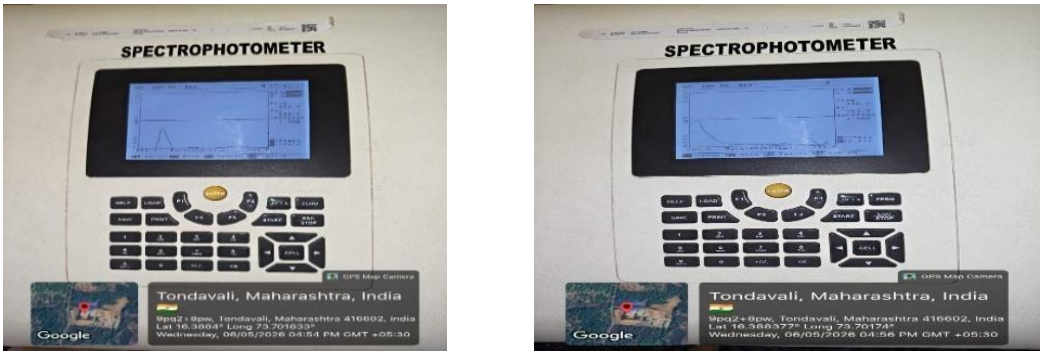


Figure No.04: UV Spectrum of Metformin and Gliclazide.

It Gives Maximum Wavelength of **Metformin At 235nm** While **Gliclazide At 226nm.**

8. Preparation of Standard Stock Solution and Dilution

8.1 Preparation of 0.1 N NaOH

1. Dissolve 4 g NaOH pellets in distilled water.
2. Make up the volume to 1000 mL with distilled water.

8.2 Preparation of Metformin and Gliclazide Stock Solutions

1. Accurately weigh 10 mg each of Metformin and Gliclazide separately.
2. Dissolve each drug in about 60 mL of 0.1 N NaOH.

Figure No.05.

3. Transfer the solutions separately into 100 mL volumetric flasks.
5. Rinse the beaker and add washings to the respective flask.
6. Make up the volume to 100 mL with 0.1 N NaOH.
7. Mix thoroughly to obtain clear and homogeneous stock solutions (100 µg/mL each).

8.3 Procedure for Dilution of Metformin and Gliclazide

1. Pipette 0.3, 0.6, 0.9, 1.2 and 1.5 mL of the 100 µg/mL stock solution into separate 10 mL volumetric flasks.
2. Make up the volume to the mark with 0.1 N NaOH. Mix thoroughly to obtain concentrations of 3, 6, 9, 12 and 15 µg/mL.
3. These diluted solutions were used for UV spectrophotometric analysis and preparation of calibration curves.

Figure No.06

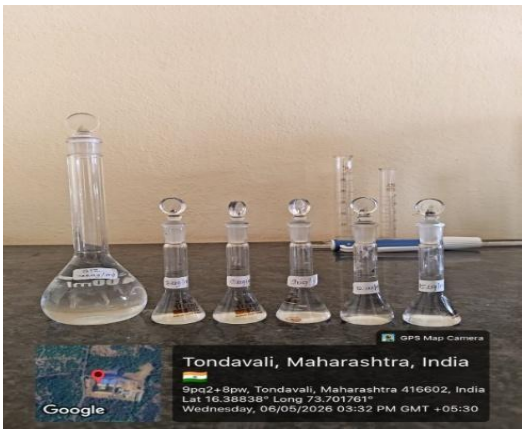


Figure No.07

8.4 Preparation of calibration curve

1. Working standard solutions of Metformin and Gliclazide were prepared from the 100 µg/mL stock solutions.
2. Dilutions of 3, 6, 9, 12 and 15 µg/mL were prepared using 0.1 N NaOH.
3. Absorbance was measured at: 235 nm for Metformin 226 nm for Gliclazide.
4. Calibration curves of Concentration vs Absorbance were plotted.
5. Regression equations were calculated for quantitative analysis.

Table No. 04

Concentration of Metformin (µg/mL)	Absorbance		Concentration of Gliclazide (µg/mL)	Absorbance	
	235nm	226nm		235nm	226nm
3	0.112	0.102	**3**	0.023	0.130
6	0.242	0.252	**6**	0.061	0.145
9	0.362	0.396	**9**	0.099	0.166
12	0.479	0.529	**12**	0.121	0.201
15	0.603	0.677	**15**	0.169	0.271

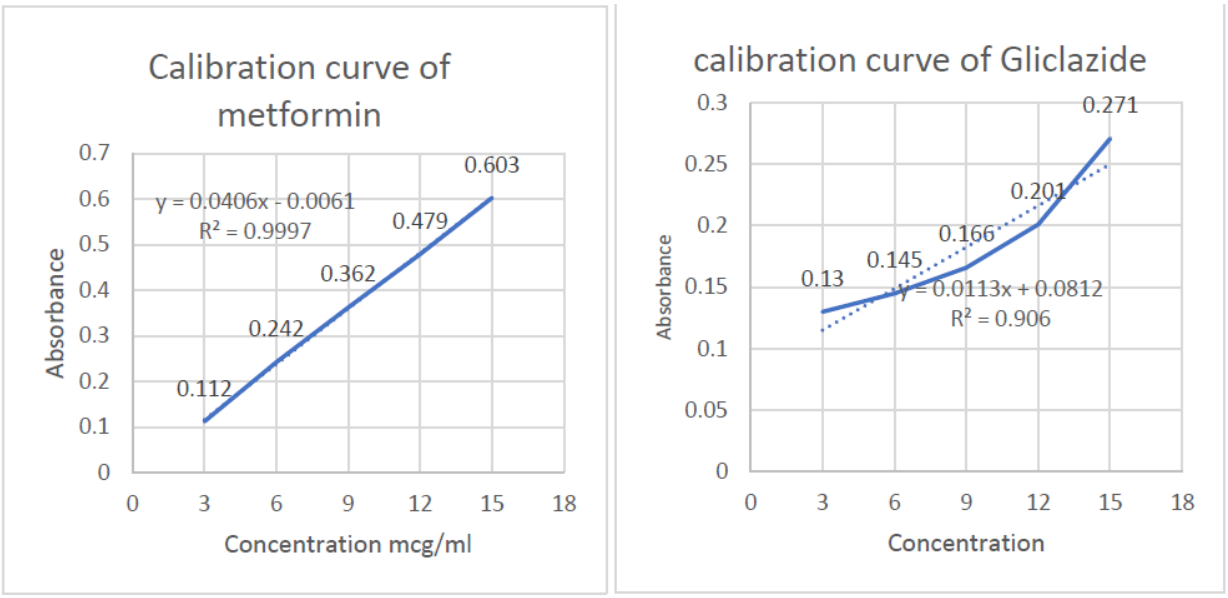


Figure No 08: Calibration Curve of Metformin & Gliclazide.

9. METHODOLOGY

Determination of Absorptivity Values both drugs at selected wavelengths:

Preparation of Mixed Sample Solution

Prepare mixture containing both drugs in suitable concentration using: Measure absorbance at:

1] 235 nm - A_1 =0.758, 2] 226 nm - A_2 =0.872

E (1%, 1cm) values of these drugs were calculated using following formula;

$$E\ (1\%,1cm) = \frac{\text{Absorbance}}{\text{Concentration (g/100mL)}}$$

Table No.05

Sr.no	Absorptivity at λ1(235 nm)		Absorptivity λ2 (226 nm)	
	Metformin	**Gliclazide**	**Metformin**	**Gliclazide**
1	0.0373	0.0076	0.0340	0.0433
2	0.0403	0.0101	0.0420	0.0241
3	0.0402	0.0110	0.0440	0.0184
4	0.0399	0.0100	0.0440	0.0167
5	0.0402	0.0112	0.0451	0.0180
Mean	**ax_1 =0.0396**	**ay_1 =0.0100**	**ax_2 = 0.0418**	**ay_2 =0.0241**

Simultaneous Equation Method

At 235nm = $a_{x1}C_x + a_{y1}C_y$ At 226nm = $a_{x2}C_x + a_{y2}C_y$

- A_1 and A_2 = absorbances at selected wavelengths

- ax and ay = absorptivity coefficients
- Cx = concentration of Metformin
- Cy = concentration of Gliclazide

Concentration of Metformin HCl

$$C_{x(MET)} = \frac{A_2a_{y1} - A_1a_{y2}}{ax2ay1 - ax1ay2}$$

Concentration of Gliclazide

$$C_{y(GLZ)} = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2}$$

Calculation

- Calculate Denominator:$(a_{x2}a_{y1} - a_{x1}a_{y2})$

$= (0.0418 \times 0.0100) - (0.0396 \times 0.0241)$

$= 0.000418 - 0.00095436$

$= -0.00053636$

- **For Metformin Concentration (C_x)**

$$C_x = \frac{(0.872 \times 0.0100) - (0.758 \times 0.0241)}{-0.00053636}$$
$$= \frac{0.00872 - 0.0182678}{-0.00053636}$$
$$= \frac{-0.0095478}{-0.00053636}$$
$$C_x = 17.80$$

- **For Gliclazide Concentration (C_y)**

$$C_y = \frac{(0.758 \times 0.0418) - (0.872 \times 0.0396)}{-0.00053636}$$
$$= \frac{0.0316844 - 0.0345312}{-0.00053636}$$
$$= \frac{-0.0028468}{-0.00053636}$$
$$C_y = 5.31$$

FINAL RESULTS

Table No. 06

Drug	Concentration
Metformin	17.80 µg/mL
Gliclazide	5.31 µg/mL

10. Method Of Validation

1. Accuracy: The degree to which the measured value closely resembles the true value is known as measurement accuracy. Three levels of each drug were studied at three concentrations (80, 100 and 120 µg/mL).

❖ **PROCEDURE**

- Preparation of Standard Stock Solutions
- Preparation of Accuracy Levels:- 80%,100% ,120%
- Preparation of 80%,100% and 120% Level Solution:-

Pipette 1 mL sample solution into 10 mL volumetric flask. Add 0.8 mL, 1.0 mL 1.2 mL Metformin stock solution. Add 0.8 mL, 1.0 mL 1.2 mL Gliclazide stock solution. Make volume up to mark with solvent. Mix properly.

Table 07: Accuracy result of metformin.

Level	Standard conc. (µg/mL	Conc. added (µg/mL)	Conc. found at 235nm (µg/mL)	% Recovery	% Mean Recovery
80%	10	8	1.616	119.52%	101.83%
100%	10	10	1.690	100%	
120%	10	12	1.744	85.99%	

Table 08: Accuracy result of gliclazide.

Level	Standard conc. (µg/mL	Conc. added (µg/mL)	Conc. found at 226nm (µg/mL)	% Recovery	% Mean Recovery
80%	10	8	1.867	123.47%	102.70%
100%	10	10	1.890	100%	
120%	10	12	1.920	84.65%	

2. Precision: The degree of agreement between a set of measurements made from repeated samplings of the same homogenous sample under specified circumstances is known as precision.

Preparation of Mixed Working Standard Solution

To prepare mixed solution containing: Metformin = 6 µg/ml and Gliclazide = 6 µg/ml.

❖ **Procedure**

1. Pipette 0.6 mL of the stock solution for metformin.
2. Pipette 0.6 mL of the stock solution of gliclazide.

3. Pour both into a volumetric flask with a capacity of 10 ml.
4. Add distilled water and 0.1 N NaOH to make up the volume.

A. Repeatability (Intraday Precision)

Make six distinct mixed standard solutions with the same concentration of gliclazide and metformin. Note the absorbance values. Determine the standard deviation, mean absorbance and percentage RSD.

B. Intermediate Precision (Interday Precision)

Repeat the same procedure on: Different day or Different analyst or Different instrument. Again, record six absorbance readings. Calculate: Mean , SD, %RSD

Table No 09: Intra-day precision and Inter-day precision results of Metformin and Gliclazide.

	INTRA-DAY PRECISION			INTERDAY PRECISION	
Sr.No	**Metformin Absorbance**	**Gliclazide Absorbance**	**Sr.No**	**Metformin Absorbance**	**Gliclazide Absorbance**
1	0.692	0.759	**1**	0.335	0.465
2	0.686	0.762	**2**	0.336	0.464
3	0.686	0.768	**3**	0.340	0.468
4	0.681	0.772	**4**	0.344	0.474
5	0.687	0.776	**5**	0.351	0.476
6	0.690	0.785	**6**	0.351	0.487
Mean	0.687	0.770	**Mean**	0.343	0.472
SD	0.012	0.0095	**SD**	0.0071	0.0086
%RSD	1.74%	1.23%	**%RSD**	2.07%	1.82%

3. Limit of Detection (LOD) & Limit of Quantification (LOQ):-

- The lowest concentration of an analyte in a sample that can be identified but may not be precisely measured under the specified experimental circumstances is known as the Limit of Detection (LOD).The LOD can be calculated using the formula.

$$LOD = \frac{3.3\sigma}{S}$$

Where

- σ= Standard deviation of the response
- S= Slope of the calibration curve
- Limit of Quantification (LOQ) is the lowest concentration of an analyte that can be quantitatively determined with acceptable precision and accuracy. The LOQ can be calculated using the formula.

$$LOQ = \frac{10\sigma}{S}$$

Where

- σ= Standard deviation of the response
- S= Slope of the calibration curve

❖ **Procedure**

1. Make a new dilution of the medication at the lowest concentration within the calibration range.

- 3 µg/mL of metformin
- 3 µg/mL of gliclazide

2. At the chosen wavelength, record absorbance six times.
3. Determine the readings' standard deviation.
4. Get the calibration curve's slope.
5. Use the formula for LOQ.

Figure No.09.

Absorbance Readings

Measure the absorbance of the low concentration solution six times at the selected wavelength.

RESULT

Table No.10

Parameter	Metformin	Gliclazide
Absorbance readings	0.164,0.159,0.162,0.161,0.162, 0.166	0.043,0.043,0.046,0.045,0.047, 0.049
Regression Equation	y = 0.0406x + 0.0061	y = 0.0113x + 0.0812
Slope (S)	0.0406	0.0113
Standard Deviation (σ)	0.00242	0.00234
LOD (µg/mL)	0.196	0.684
LOQ (µg/mL)	0.596	2.07

The Limit of Quantitation (LOQ) is the lowest concentration of analyte that can be quantified with acceptable precision and accuracy, while the Limit of Detection (LOD) is the lowest concentration that can be detected but may not be accurately quantified, according to ICH Q2(R1) guidelines. Using the current UV spectrophotometric technique. The low LOD and LOQ values show that the new analytical technique has good sensitivity and can successfully detect and quantify very tiny amounts of both pharmaceuticals.

4. Ruggedness

The reproducibility of outcomes when the approach is used in real-world scenarios is known as ruggedness. The degree to which test results from various situations (different labs, different analysts, different tools) are consistent.

❖ **Procedure**

1. Standard Stock Solution preparation.
2. Mixed Working Standard Solution Preparation.
3. Fill a 10 mL volumetric flask with a pipette.
4. 1.0 mL of Metformin stock solution.
5. Gliclazide stock solution (5.1.0 mL).
6. Use NaOH solvent to increase the volume to 10 mL.
7. Analysis by Various Analysts.
8. Under identical experimental settings, Analysts 1 and 2 examine the created mixed solution and Calculate: Mean, Standard deviation (SD) ,% Relative Standard Deviation (%RSD) Acceptance Criteria:- As per ICH:%RSD should be less than 2%.

Table No. 11.

Sr.No.	Parameter	Analyst 1	Analyst 2	Mean	SD	%RSD
1	**Metformin**	0.539	0.548	0.543	0.00636	1.17
2	**Gliclazide**	0.283	0.286	0.284	0.00212	0.74

The low %RSD values (<2%) indicate that the developed UV spectrophotometric method is rugged and reproducible under normal operating conditions.

5. Robustness

Robustness is a statistic that characterizes an analytical procedure's performance under normal operating conditions and its resistance to small, deliberate changes in method parameters.

❖ **Procedure**

1. Preparation of Standard Stock Solution.
2. Preparation of Mixed Working Standard Solution.
3. Pipette into 10 mL volumetric flask.
4. 1.0 mL of Metformin stock solution.
5. 1.0 mL of Gliclazide stock solution.
6. Make up volume to 10 mL using NaOH solvent.
7. Measure absorbance at: Nominal wavelength, slightly lower wavelength, slightly higher wavelength.
8. Record absorbance and Calculate: 1. Mean, SD, %RSD.

Table No. 12

	Metformin			**Gliclazide**	
Sr.No.	**Wavelength (nm) MET**	**Absorbance**	**Sr.No.**	**Wavelength (nm) GLZ**	**Absorbance**
1	233	0.550	**1**	224	0.291
2	235	0.547	**2**	226	0.289
3	237	0.542	**3**	228	0.287
Mean		0.546	**Mean**		0.289
SD		0.0040	**SD**		0.0020
%RSD		0.73%	**%RSD**		0.69%

The analytical method is regarded as robust since the %RSD results for both medications are below the ICH-recommended acceptable limit of 2%.

10. RESULT

Using 0.1 N NaOH as the solvent, a straightforward, quick, accurate, precise and cost-effective UV spectrophotometric technique was successfully established for the simultaneous measurement of metformin hydrochloride and gliclazide in bulk dosage form. The technique used absorbance measurements at 235 nm for metformin and 226 nm for gliclazide and it was based on the simultaneous equation method. Both drugs obeyed Beer–Lambert's law in the concentration range of 3–15 µg/mL with good linearity. The regression equation for Metformin was found to be

y = 0.0406x + 0.0061 and for Gliclazide: **y = 0.0113x + 0.0812**

The new method was validated for a number of analytical characteristics, including accuracy, precision, linearity, ruggedness, robustness, LOD and LOQ, in accordance with ICH requirements. The method's correctness was demonstrated by the percentage recovery

numbers, which were found to be within acceptable bounds. Good repeatability and intermediate precision were confirmed by precision studies with %RSD values less than 2%. The LOD and LOQ values obtained were

Metformin: LOD = 0.196 µg/mL

LOQ = 0.596 µg/mL

Gliclazide: LOD = 0.684 µg/mL

LOQ = 2.07 µg/Ml

Ruggedness and robustness studies also showed %RSD values below 2%, indicating that the method is reproducible and unaffected by small variations in analytical conditions.

The simultaneous equation method successfully estimated the concentration of both drugs in the mixed sample solution:

- Metformin = 17.80 µg/mL
- Gliclazide = 5.31 µg/mL

Hence, the developed UV spectrophotometric method was found to be reliable and suitable for routine quantitative analysis of Metformin Hydrochloride and Gliclazide in bulk and pharmaceutical dosage forms.

11. CONCLUSION

Using the simultaneous equation technique, the current work successfully developed and validated a straightforward, sensitive, quick and economical UV spectrophotometric method for the simultaneous estimation of metformin hydrochloride and gliclazide in bulk dose form. The devised approach complies with Beer-Lambert's law and demonstrated high linearity within the chosen concentration range. The method's accuracy, precision, robustness and ruggedness were verified by validation experiments conducted in accordance with ICH recommendations. The method's great sensitivity for both drug detection and quantification were demonstrated by the low LOD and LOQ values. The procedure is straightforward and cost-effective because it uses distilled water and 0.1 N NaOH as the solvent system. The precision, ruggedness and robustness experiments' %RSD values were within allowable bounds, demonstrating the method's dependability. Thus, in both research and industrial facilities, the suggested UV spectrophotometric approach can be effectively used for routine quality control analysis and simultaneous estimate of metformin hydrochloride and gliclazide in bulk and pharmaceutical dose forms.

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