DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHODSFOR SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND GLIMEPIRIDE IN PURE AND TABLET DOSAGE FORM

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Abstract

The present work's objective was to establish an accurate, straightforward, precise, and trustworthy method for estimating metformin hydrochloride and Glimepiride in their combination dosage form. The method for the simultaneous estimation of metformin hydrochloride and Glimepiride has been developed based on the simultaneous equation method at two selected wavelengths of 237nm and 228nm, respectively, and also the absorbance ratio method at two selected wavelengths of 232nm (Iso-absorptive point) and 228nm (λmax of Glimepiride). For metformin hydrochloride and Glimepiride, the concentration range of 5-25 ug/ml or 5-25ug/ml, respectively, was used to achieve linearity,LOD values were 0.74ug/ml and 0.79ug/ml, LOQ values were 2.44ug/ml and 2.37ug/ml, and recovery (accuracy test) values ranged from 99.43 to 99.98, respectively. The precision test indicator for both drugs, the relative standard deviation (RSD), was less than 2%. Metformin HCL and Glimepiride in commercial dose form were effectively determined using the methodology.

Keywords: Metformin HCL, Glimepiride, Simultaneous estimation, Absorption Ratio,Area Under Curve, Accuracy, Linearity, Recovery, Precision.

Introduction

Clinically, metformin HCL (MET) N With a molecular weight of 165.62 g/mol2, the anti-diabetic medication dimethyl amidodicarbonimidicdiamide hydrochloride, belongs to the biguanide class and is used in the treatment of type 2 diabetics. Metformin HCL is largely insoluble in acetone, ether, and chloroform but easily soluble in water. Metformin has a Pka of 12.4. Metformin HCL in a 1% aqueous solution has a pH of 6.6. Metformin is a BCS class III agent with high solubility and low permeability. The main effects of Metformin included improving insulin sensitivity by raising peripheral glucose absorption and utilisation and boosting glucose transport across cell membranes in skeletal muscle.

Fig.1 Structure of Metformin hydrochloride

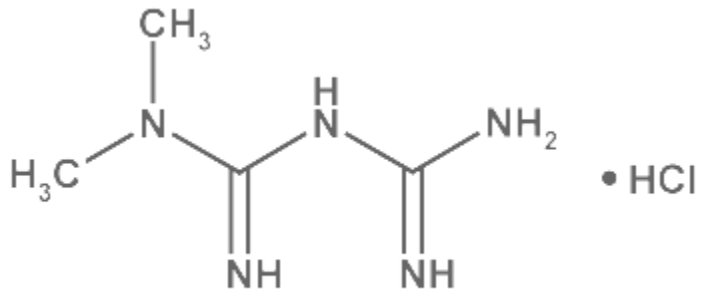
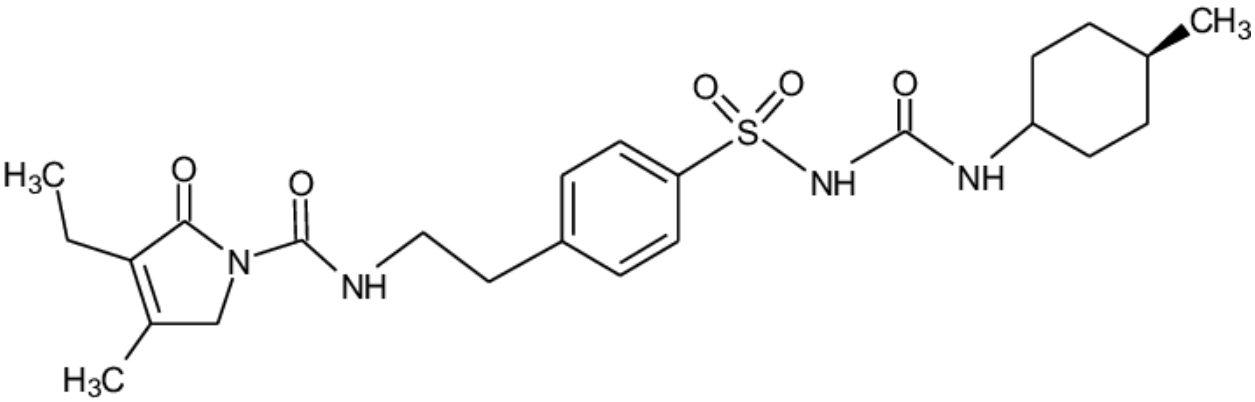


Fig.2 Structure of Glimepiride



The mechanism of action for Glimepiride is [3-ethyl-4-methyl-N-[2-4-([(4-methyl cyclohexyl)carbamoylamino]sulfonyl)phenyl] [2-oxo-2,5-dihydro-1 H pyrrole-1-carboxamide] It appears that reducing blood sugar depends on triggering the release of insulin from active pancreatic beta cells and improving the sensitivity of peripheral tissues to insulin. Glimepiride, which belongs to the BCS II class of drugs with poor solubility and high permeability, has a molecular weight of 490.6 and is virtually insoluble in water. The potassium conductance is decreased, and the membrane becomes depolarised when Glimepiride binds to an ATP-sensitive potassium channel receptor on the surface of pancreatic cells. Voltage-sensitive calcium channels are stimulated by membrane depolarisation to increase calcium ion inflow. As the intracellular calcium ion concentration rises, insulin is secreted.

The simultaneous measurement of the dose forms of glimepiride and metformin HCL was also reported, like UV spectroscopy. As a result, an effort was made to create a new, quick, and sensitive UV spectrometric approach and validate it per ICH requirements. A thorough literature review reveals the absence of a spectrophotometric analytical method for simultaneously measuring metformin HCL and Glimepiride in pharmaceutical formulations. The current effort aimed to create an accurate, straightforward, exact, and dependable approach for estimating these two medications in their combined dosage form.

MATERIALS AND METHODS

1. Apparatus and Instrumentation

All spectral measurements were conducted using a UV probe in conjunction with a double beam spectrophotometer (Jasco V 630), a Shimadzu model 1700 with a 1 nm spectral bandwidth, 0.3 nm wavelength precision, and a few 10 mm matching quartz cells. The weighing was done using a single-pan electronic balance, and an ultrasonic cleaning bath was used to sonicate the solution. The validation investigation made use of calibrated volumetric glasses.

2. Materials

Glimepiride API and metformin HCL reference standard. The commercial formulation Glycomet-GP1, whose label states each tablet contains 500 mg of metformin hydrochloride and 1 mg of Glimepiride, was bought from a store in Ahmednagar.

3. Method development

Preparation of standard stock solution

A stock solution was generated by diluting 10 mg of each medication in enough methanol in a separate volumetric flask and then bringing the volume to 100 ml to give each drug a 100 ug/ml concentration. For the final concentration, dilutions of this stock solution were made between 5 and 25 ug/ml for Glimepiride and 5 to 25 ug/ml for metformin HCL, and they were scanned between 200 and 400 nm. As a control solution, methanol was utilised.

Method:

Application of the proposed Method for the determination of MET and GLIM in Tablet Dosage Form:

1) Simultaneous Estimation Equation:

The average weight of 20 tablets was determined by weighing them all. To create the powder, the tablets were crushed. A 100 ml volumetric flask was filled with tablet powder equal to the fill weight of one tablet, and it was then ultrasonically processed for 10 minutes. After that, a Whatmann filter paper (No.41) was used to filter the resultant solution. A final concentration of 20ug/ml was obtained by dilution of the aliquot with methanol. The concentration of both drugs was ascertained by measuring the sample's absorbance in spectrum mode at 228.0 nm and 237.0 nm, respectively. The concentration was then calculated using the corresponding formulas.

$$Cx = A2ay1 - A1ay2 / ax2ay1 - ax1ay2$$

$$Cy = A1ax2 - A2ax1 / ax2ay1 - ax1ay2$$

Where,

Cx = Concentration of Metformin,

Cy = Concentration of Glimepiride;

A1 = absorbance of mixture at 228nm;

A2 = absorbance of mixture at 237nm;

ax1 = Absorptivity of Met at 228nm;

ax2 = Absorptivity of Met at 237nm;

ay1 = Absorptivity of Glim at 228nm;

ay2 = Absorptivity of Glim at 237nm.

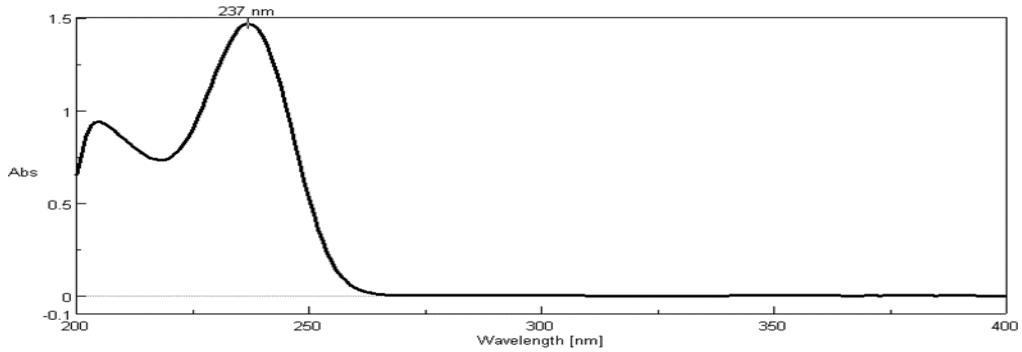


Fig 1: It shows λmax of Metformin HCL

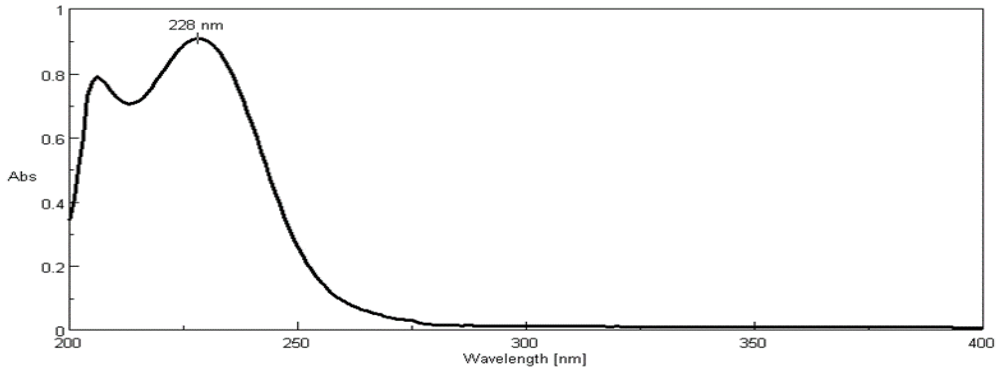


Fig 2: It shows λmax of Glimepiride

2) Absorbance Ratio Method:

The wavelengths 232.0 nm (the iso-absorptive point) and 228.0 nm (the maximum absorption of Metformin) were chosen for examination in the absorbance ratio method from the drug's overlay spectrum (fig. 3). Both medicines' absorptivities at both wavelengths were calculated. The concentration of each component in the sample was determined using the following set of equations,

Cx = Qm-Qy/Qx-Qy.A1/ax1.....(1);

Cy = Qm-Qx/Qy-Qx.A1/ay1.....(2)

Where

Cx = Concentration of Metformin,

Cy = Concentration of Glimepiride,

A1 = absorbance of sample at 228nm,

ax1 = absorptivity of Glimepiride at 228nm,

ay1 = absorptivity of Metformin at 237nm,

Qm = ratio of absorbance of sample solution at 232nm and 228,

Qx = ratio of absorptivity of Metformin at 232nm and 228,

Qy = ratio of absorptivity of Glimepiride at 232nm and 237nm.

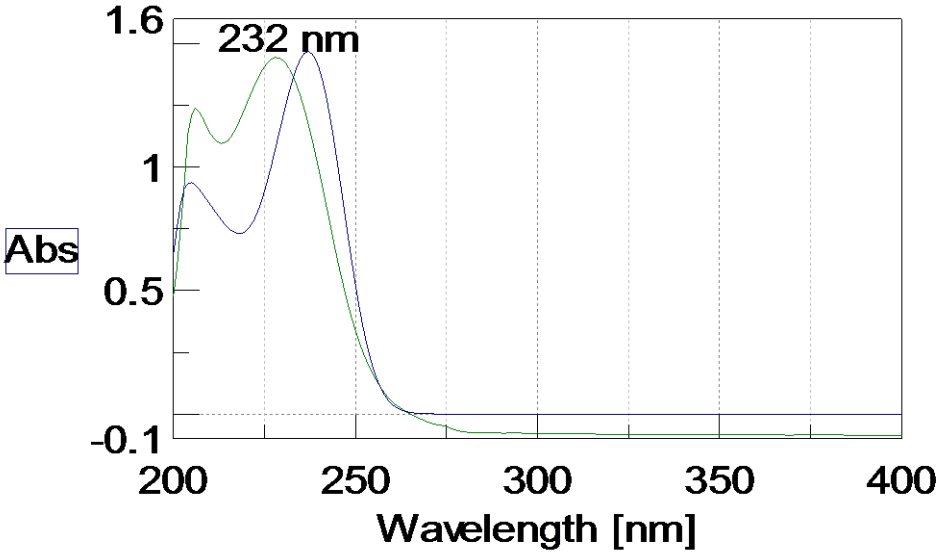


Fig 3: Isobestic point of Metformin HCL and Glimepiride

3) Area Under Curve Method

Standard Glimepiride and Metformin HCL solution get Area under curve between 237 and 228 nm, which were individually scanned to obtain the drug's spectra, were chosen for the analysis. The metformin HCL and glimepiride calibration curves were constructed in the 5–25 ug/ml concentration range at their respective AUC ranges. Both drugs showed Beer Lambert's Law behaviour within the specified concentration range. Table 2 provides the method's slope, intercept, and coefficient of correlation (r) values.

Area calculation: $(\alpha + \beta) = \int_{\lambda 2}^{\lambda 1} A d\lambda$

Where,

a = Area of portion bounded by curve data and a straight line correcting the start and end point,

β = the area of portion bounded by a straight line correcting the start and end point on the curve data and horizontal axis,

λ1 and λ2 are the curve region's wavelength range start and endpoints.

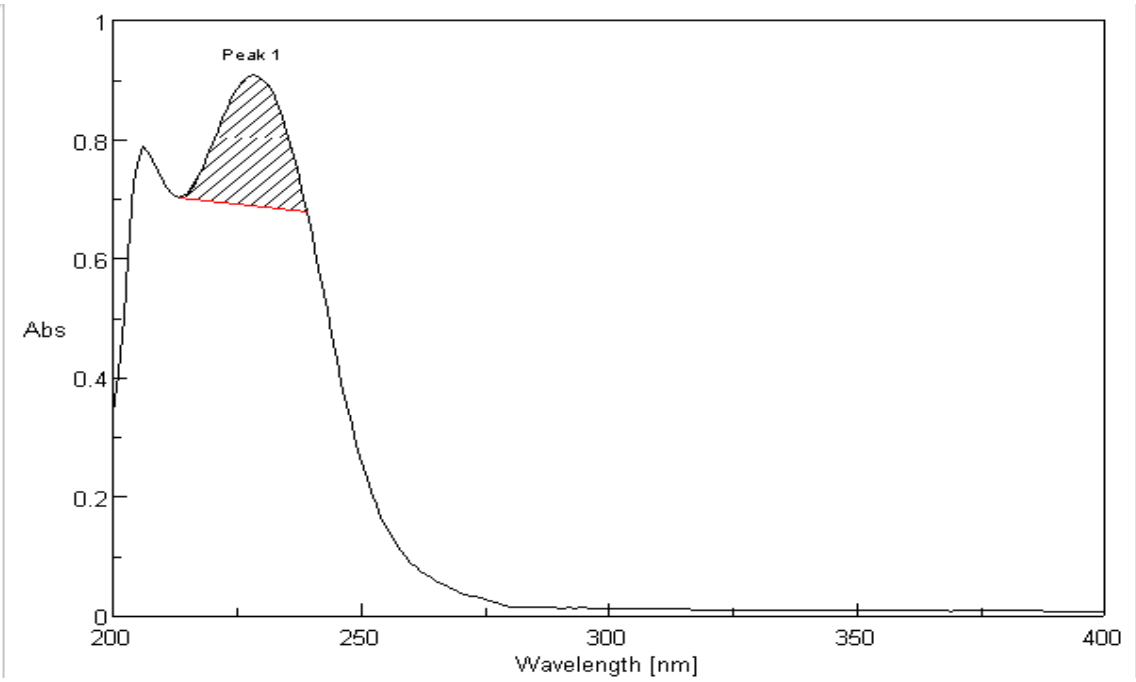


Fig 4: It shows the AUC of Metformin HCL

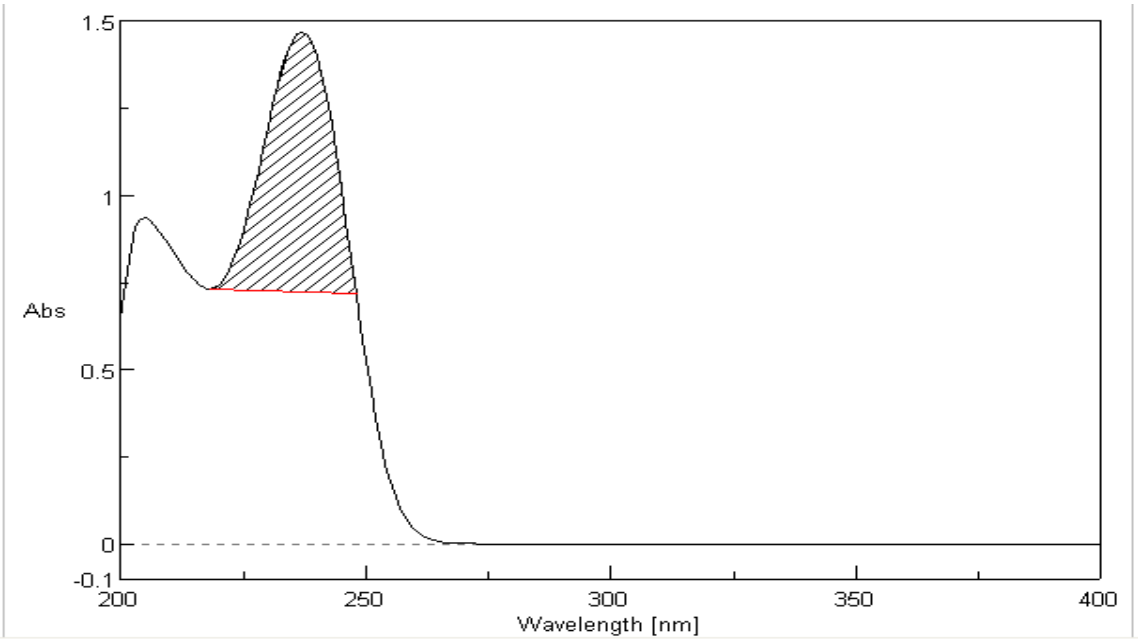


Fig 5: It shows the AUC of Glimepiride

Application of the proposed methods for the determination of Metformin HCL and Glimepiride in the Tablet dosage form

Twenty tablets were weighed, and the weight of that many tablets was transferred to a 50 ml volumetric flask. It was ultrasonically processed for 20 minutes before the volume was poured

with methanol. After that, a Whatmann filter paper was used to filter the solution (NO.42). The filtrate was diluted as necessary.

Table 1: Table shows the result of Analysis of Tablet Formulation

Method	Drug	Label Claim mg	Sample Solution Concentration (ug/ml)	Amount Found(%)*±	% Recovery	%RSD
A	Metformin HCL	500mg	20	101.29	101.54	0.6394
B	Metformin HCL	500mg	20	99.69	101.96	
A	Glimepiride	1mg	20	100.18	100.69	0.6481
B	Glimepiride	1mg	20	99.47	99.07	

The absorption maxima method measured the sample's absorbance at 237 nm for metformin HCL and 228 nm for Glimepiride, respectively, in zero-order spectrum modes. The calibration curve was used to calculate the sample solution's concentration.

In the AUC Method, the area under the curve in the 217–247 nm and 213-239 nm wavelength ranges was used to calculate the concentration. The concentration of the sample solution was determined using the calibration curve.

Validation Parameters:

1) Accuracy:

A systemic error was a factor in determining the accuracy of an analysis. Accuracy is given as a percentage of recovery from the test of a known, additional amount of analyte. Accuracy has noted that for the two medications, the RSD percentage is less than 2%, which is acceptable.

2) Linearity:

The linearity of these medications was examined by statistically calculating the response of the analyte and the concentration. Results must be expressed as correlation coefficients.

For first-order derivatives, spectrophotometric methods, the calibration curve regression equations for Metformin HCL and Glimepiride were y= 0.0873x+0.1157 (r2 = 0.999) at 237 nm and y= 0.0608x+0.0314 (r2 = 0.999) at 228 nm, respectively. The calibration curve was plotted as shown below using concentration ranges of 5–25 ug/ml for Glimepiride and 5–25 ug/ml for Metformin.

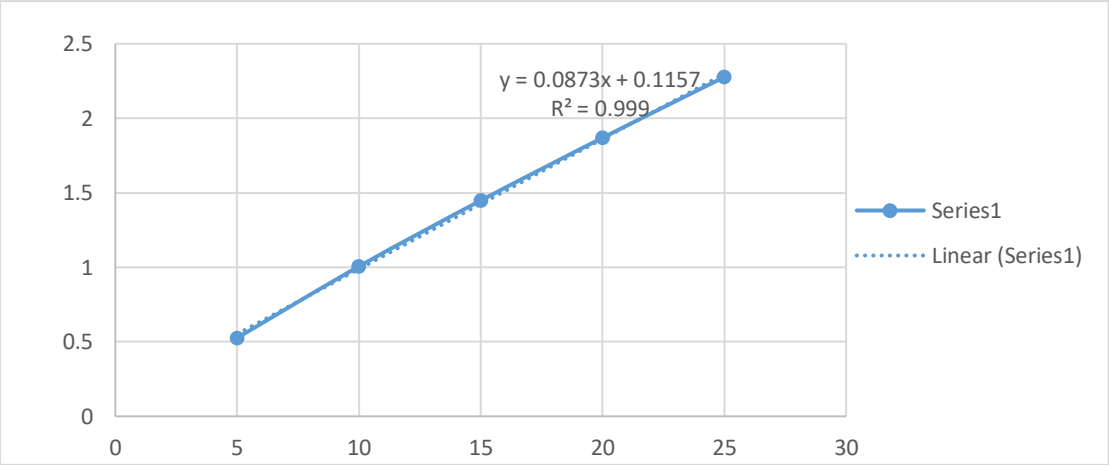


FIG 6: LINEARITY OF METFORMIN HCL

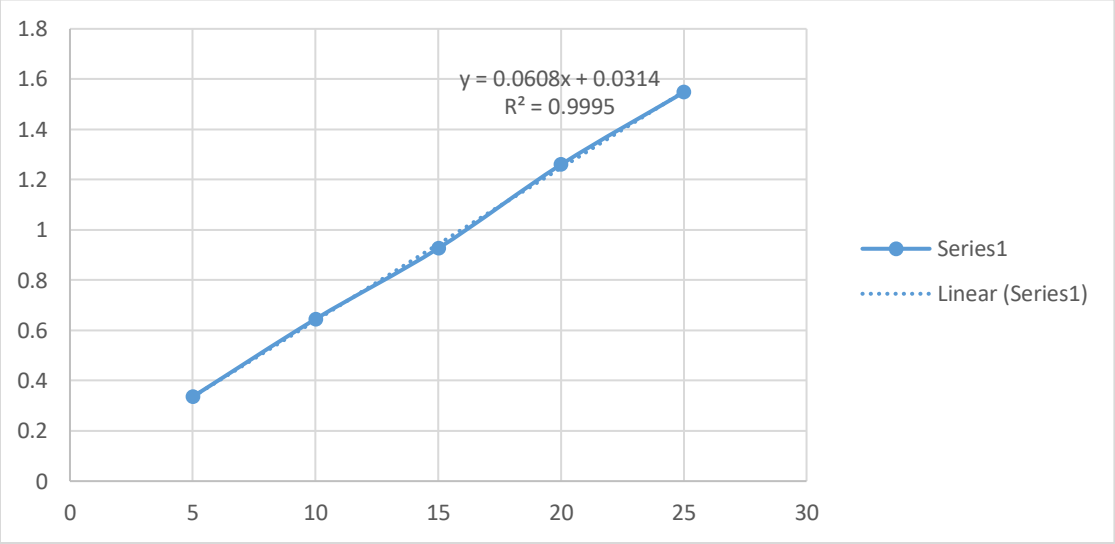


FIG 7: LINEARITY OF GLIMEPIRIDE

3)**% Recovery:**

A range of percentage concentrations was injected to examine the analyte's recovery in the formulation.

3) Assay:

Assay refers to examining the marketed formulation compared to the reference solution.

4) Precision:

To test the proposed method's reproducibility, tablet assays were run in the morning, afternoon, and evening on the same day (intra-day assay precision) and on three different days (Inter-day precision). Results for intra-day and inter-day precision are presented as a percentage of RSD.

6) Robustness:

The robustness of the proposed approaches was evaluated by adjusting variables like the wavelength range and slit width. None of these factors substantially impacted the medicines' absorbance, demonstrating the robustness of the suggested approaches.

5) Ruggedness:

By examining aliquots from homogeneous slots in several laboratories by different analysts under similar operational and environmental conditions, the robustness of the suggested methodologies was evaluated.

6) Sensitivity:

Using the formulas LOD = 3xσ/S and LOQ = 10xσ/S, the limits of detection (LOD) and quantification (LOQ) were calculated.

Where

σ = the intercept's standard deviation,

S stands for "the slop,"

Metformin HCL's LOD and LOQ were discovered to be 0.74ug/ml and 2.44ug/ml, respectively, and Glimepiride's LOD and LOQ were determined to be 0.79ug/ml and 2.37ug/ml.

Table 2: Optical Characteristics and Precision

Sr. No.	Parameter	MetforminHCL	Glimepiride
1	λ range	200-400	200-400
2	Regression Equation (y=mx +c)	y=0.0873x+0.1157	Y=0.0608+0.0314
3	Measured Wavelength	237	228
4	Linearity range	5-25ug/ml	5-25ug/ml
5	Slope	0.0873	0.0608
6	Intercept	0.1157	0.0314
7	Correlation Coefficient(R^2)	0.999	0.999
8	Limit of Detection(LOD) ug/ml	0.7482	0.7905
9	Limit of Quantitation(LOQ)ug/ml	2.4493	2.3719

Table 3: Result of Drug content and analytical recovery of Metformin HCL and Glimepiride

Excess drug added to the analyst (%)	Drug	%Recovery		%RSD	
		Method A	Method B	Method A	Method B
80	Metformin HCL	98.28	100.58	0.0615	0.0601
100		100.42	101.73	0.0602	0.0595
120		99.02	94.02	0.0611	0.0643
80	Glimepiride	98.14	100.09	0.0304	0.0298
100		101.02	100.92	0.0296	0.0296
120		99.73	99.98	0.0299	0.0299

Table 4: Results of Intra-day and Inter-day Precision

Method	Drug	Intra-day Precision		Inter-day Precision	
		SD	%RSD	SD	%RSD
A	Metformin HCL	0.774	0.398	0.681	0.381
B		0.785	0.428	0.589	0.265
A	Glimepiride	0.248	0.845	0.142	0.099
B		0.198	0.745	0.187	0.107

Result and Discussion

The methods covered in this paper offer a practical and precise method for the investigation of glimepiride and metformin HCL in their bulk and pharmaceutical dosage forms. Glimepiride and metformin HCL absorbance maxima of 228 and 237 nm, respectively, were chosen for the analysis. For Metformin HCL and Glimepiride, linearity for detector response was seen in the 5–25 ug/ml concentration range. In tablet analysis, the percentage amounts for glimepiride and metformin HCL were determined to range from 101.29 to 99.69 to 100.18 to 99.47. (Table.1). The tablet formulation's standard deviation and coefficient of variation were determined to be less than 2.0, demonstrating the accuracy of the procedures. Recovery studies were used to determine the suggested method's accuracy, and the results are reported as a percentage recovery. Metformin HCL and Glimepiride showed a % recovery in the range of 100.23 and 99.67% values of standard deviation, and a satisfactory low coefficient of variation, demonstrating the correctness of all the procedures. For Metformin HCL and Glimepiride, respectively, the % RSD for Intraday Assay Precision was found to be 0.398 and 0.428 for

Method A and B. It was discovered that the LOD and LOQ for Glimepiride and Metformin HCL were 0.79ug/ml and 2.37ug/ml and 0.74ug/ml and 2.44ug/ml, respectively. Based on the results, it can be concluded that the suggested approach is accurate, precise, repeatable, and cost-effective and may be used for routine quality control of Metformin HCL and Glimepiride in bulk medication and its pharmaceutical.

Conclusion:

For Metformin HCL and Glimepiride, different UV spectrometric methods were established in bulk and tablet dosage forms using the absorbance maxima and area under the curve approaches. Additionally, bulk and combination dose forms for the simultaneous measurement of metformin HCL and Glimepiride were used with UV Spectrometric techniques. According to ICH criteria, the procedures were validated. The calculated standard deviation and percent RSD for these procedures are less than 2, showing that the approaches have high precision. The outcomes of the recovery studies demonstrated the great level of precision of these techniques. In conclusion, the established methods can be successfully used to estimate the concentrations of glimepiride and metformin HCL in bulk and pharmaceutical dosage form since they are accurate, precise, and selective.

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