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# The Development And Verification Of A UV-Visible Spectrophotometric Technique For Glipizide Evaluation

**AUTHORS**

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## ABSTRACT

Glipizide is an oral hypoglycemic medication that works by inhibiting the ATP-sensitive K channel, which stimulates the release of insulin from pancreatic beta cells. Glipizide's creation and verification using UV spectrophotometer use. Glipizide has a concentration range of 1–5µg/ml and follows Beer's law, having a maximum absorbance at 275 nm. Recovery checking was used to test the three distinct accuracy concentrations: 80%, 100%, and 120%. The recovery % was discovered to be comfortably within the allowed range. The interday % R.S.D. for 10, 20, and 30µg/ml was 0.006, 0.00325, and 0.752, respectively. The intraday %RSD for 80% , 100% , 120% was 0.243, 0.325, 0.181 respectively . For linearity the concentration range is 10, 20 ,30 ,40 , and 50µg/ml .

## KEYWORDS

Glipizide , Spectroscopic Technique , Method Validation ,ICH Guidelines

## Introduction:

Glipizide is one of the most widely recommended medicines for type 2 diabetes treatment. It's a sulfonylurea-based oral hypoglycemic drug. It is a property of second-generation sulfonylurea that it is active at very low doses. Glipizide lowers blood glucose via increasing insulin secretion and modifying insulin sensitivity; most patients experience a persistent rise in glucose stimulated insulin secretion throughout long-term treatment.[1]

A significant amount of the antidiabetic impact of glipizide is also thought to be derived from changing how responsive insulin-sensitive tissues are, which amplifies the effects of insulin (3,4). Studies have indicated that glipizide can enhance long-term effects on insulin secretion (4). However, other research using glipizide for longer than six months has not shown tolerance to the hypoglycemia effects (5). Glipizide medication was shown to stabilize blood glucose levels for at least 4 years (5). According to one study, 70% of patients on glipizide maintained control for up to 6 years7 (with a cumulative secondary failure rate of about 30%)(4-5). Additionally, data indicate that glipizide enhances glucose consumption through increasing the number of insulin receptors and/or extrapancreatic availability of insulin, in addition to stimulating pancreatic insulin secretion(6). Several investigations, including meta-analyses, showed that sulfonylurea supplementation

reduces daily insulin dose by 25–35% while improving glycemic control [7-8]. According to the literature currently in publication, analytical techniques such as gas chromatography (GC) and high performance liquid chromatography (HPLC) have been developed for the purpose of determining the presence of glipizide in human serum, plasma, or urine.[9,10,11,12]. The chemical name (IUPAC) of Glipizide is *N*-[2-[4-(cyclohexylcarbamoylsulfamoyl)phenyl]ethyl]-5-methylpyrazine-2-carboxamide with molecular formula $C_{21}H_{27}N_5O_4S$ and molecular weight is 445.5.[2 ]

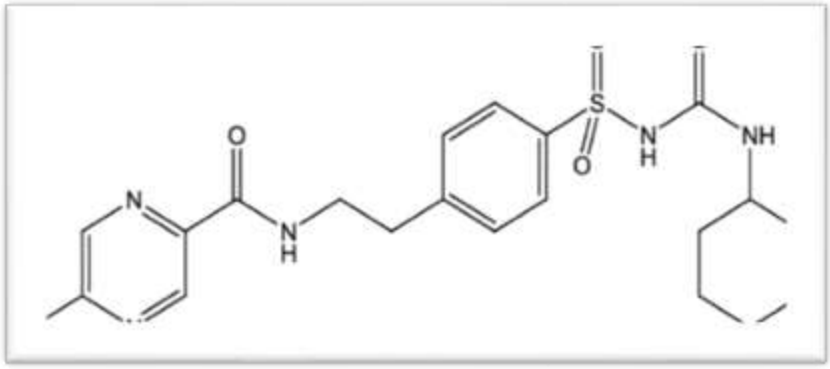


Fig.No.1 Chemical Structure of Glipizide

The goal of this research is to develop a simple, fast, selective, and cost-effective UV spectrometric method for quantifying Glipizide in bulk and pharmaceutical dose form. The method was shown in accordance with ICH Q2(R1) standards. As per the literature survey, it is revealed that some HPLC method[13,14,15,16]were reported,few HPTLC method [17,18]work was found and liquid chromatography work has perform earlier[19]. In the present study the attempt was made to develop and validate a cost effective UV Spectroscopic method for the estimation of Glipizide.

## MATERIALS AND METHODS:

### Materials used

Pure standard Glipizide was obtained as a gift sample from Ajanta Pharma Ltd. Mumbai. Commercial tablet of Glipizide formulation was purchased as research sample from Wockhardt Ltd, Aurangabad. DMF is used as a solvent.

## INSTRUMENTATION:

UV-Visible Double Beam Spectrophotometer (Systronics-2201) with 1 cm matches quartz cell, electronic balance (SHIMADZU-AY220) and a sonicator (Oscar Ultrasonic Cleaner Microclean-103) was used in the study.

UV-Visible Double Beam Spectrophotometer

| Sr.No. | Parameter | Optimized Method |
|---|---|---|
| 1 | Model No. | UV-1800 |
| 2 | Cuvvete | Quartz Cuvette |
| 3 | Detector | UV-Visible Detector |
| 4 | Scan mode | Single |

| | | |
|---|---|---|
| 5 | Scan Range | 200-400 |
| 6 | λ max | 290 |
| 7 | Software | UV-Probe |

**Preparation of standard stock solution**

Standard stock solution of Glipizide (GLP) was prepared by dissolving 10 mg GLP in 100 ml of 0.1N NaOH to obtain concentration of 100µg/ml. Spectrum measurement of Glipizide in 0.1N NaOH.

The second stock solution was prepared by diluting 0.5 ml of the above standard stock solution upto10 ml and scanned between 200- 400 nm in UV Visible double beam spectrophotometer. The UV absorption spectrum of Glipizide showed peak at 229 nm. The maximum wavelength of 229 nm was selected for the present study.

**Assay**:

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 10 mg of glipizide transferred into 10 ml of volumetric flask and dissolved in NaOH. This solution was sonicated and the final volume was made up to the mark with 0.1N NaOH. 1 ml of solution was transferred into 10 ml volumetric flask and adjust upto the mark. The absorbance of this solution measured at 229 nm. The result of assay was shown in table 1.

**Method validation**

Validation parameter such as Linearity and range, Accuracy, precision, ruggedness, robustness, LOD and LOQ according to ICH Q2(R1) guideline.

**Accuracy**

Accuracy was determined by preparing solution of different concentration that is 80%, 100%, 120%. The percentage recovery was calculated (table 2).Three saple solution at each level were tested for absorbance 275 nm in camparision to blank . the drug's absorbance at each level was used to compute the percent recovery of the drug at each of three level . recovery experiments were conducted at 80, 100 , 120 % of the target concentration , respectively , to determine the accuracy of the suggested approach .using the current technique , a predetermined quantity of pre-analyzed sample solution was spiked with standard glipizide solution 10µg/ml.

**Precision**

The precision (measurement of intra-day, inter-day) determined by analyzing the five samples of same concentration (30 µg/ml) the absorbance was noted. From the measured absorbance result mean, standard deviation was then computed **Limit of Detection (LOD).**The limit of detection (LOD) was determined by using linearity. LOD was calculated by using equation-

$$LOD = 3.3\ \delta/s$$

Where δ is a standard deviation and s is the slope.

**Linearity**:

Aliquots of 0.1, 0.2, 0.3, 0.4, 0.5 were taken from standard stock solution and the volume made upto10 ml with 0.1N NaOH. Calibration curve was plotted between absorbance versus concentration. By graphing absorbance on y-axis against concentration [ 10 to 50] on x- axis a calibration curve was created .

| Sr. no | Concentration [GLP] | Absorbance |
|---|---|---|
| 1 | 10 | 0.411 |
| 2 | 20 | 0.774 |
| 3 | 30 | 1.101 |
| 4 | 40 | 1.422 |
| 5 | 50 | 1.822 |

Table 1- Linearity Study of Glipizide

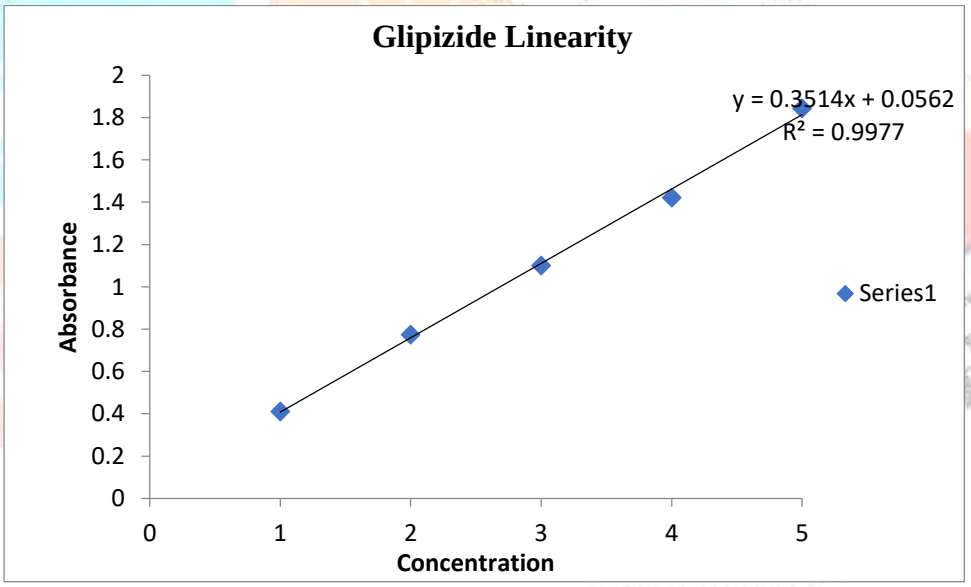


Fig 1– Linearity Curve for Glipizide

**Precision (Table No.3)**

**1] INTER DAY PRECISION**

**For Glipizide :**

| Sr no | Concentration | Interday | Absorbance | Mean | SD | RSD |
|---|---|---|---|---|---|---|
| 1 | 10µg/ml | Day1 | 0.411 | 0.421667 | 0.002887 | 0.00699 |
| 2 | 10µg/ml | Day2 | 0.416 | | | |
| 3 | 10µg/ml | Day3 | 0.411 | | | |

Table – Inter-Day Precision for Glipizide [10µg/ml]

| Sr no | Concentration | Interday | Absorbance | Mean | SD | RSD |
|---|---|---|---|---|---|---|
| 1 | 20µg/ml | Day1 | 0.774 | 0.773667 | 0.002517 | 0.00325 |
| 2 | 20µg/ml | Day2 | 0.776 | | | |
| 3 | 20µg/ml | Day3 | 0.771 | | | |

Table – Inter-Day Precision for Glipizide[20µg/ml]

| Sr no | Concentration | Interday | Absorbance | Mean | SD | RSD |
|---|---|---|---|---|---|---|
| 1 | 30µg/ml | Day1 | 1.101 | 1.103666 | 0.41029 | 0.752577 |
| 2 | 30µg/ml | Day2 | 1.106 | | | |
| 3 | 30µg/ml | Day3 | 1.104 | | | |

Table – Inter-Day Precision for Glipizide[30µg/ml]

## 2] INTRA- DAY PRECISION

**For Glipizide :**

| Sr no | Concentration | Intraday | Absorbance | Mean | SD | RSD |
|---|---|---|---|---|---|---|
| 1 | 10µg/ml | Morning | 0.411 | 0.41 | 0.001 | 0.243 |
| 2 | 10µg/ml | Afternoon | 0.409 | | | |
| 3 | 10µg/ml | Evening | 0.410 | | | |

Table – Intraday Precision at 10µg/ml

| Sr no | Concentration | Intraday | Absorbance | Mean | SD | RSD |
|---|---|---|---|---|---|---|
| 1 | 20µg/ml | Morning | 0.774 | 0.773 | 0.0025 | 0.325 |
| 2 | 20µg/ml | Afternoon | 0.771 | | | |
| 3 | 20µg/ml | Evening | 0.776 | | | |

Table – intraday precision at 20µg/ml

| Sr no | Concentration | Intraday | Absorbance | Mean | SD | RSD |
|---|---|---|---|---|---|---|
| 1 | 30µg/ml | Morning | 1.101 | 1.103 | 0.002 | 0.181 |
| 2 | 30µg/ml | Afternoon | 1.103 | | | |
| 3 | 30µg/ml | Evening | 1.105 | | | |

Table – intraday precision at 30µg/ml

**Repeatability**

**For Glipizide**

| Sr no | Concentration µg/ml | Absorbance |
|---|---|---|
| 1 | 10 µg/ml | 0.411 |
| 2 | 10 µg/ml | 0.412 |
| 3 | 10 µg/ml | 0.411 |
| 4 | 10 µg/ml | 0.412 |
| 5 | 10 µg/ml | 0.416 |
| 6 | 10 µg/ml | 0.417 |

| Mean | SD | RSD |
|---|---|---|
| 0.413167 | 0.002639 | 156.561955286 |

Table- Repeatability study of Glipizide

**Range**

The range of an analytical procedure is an interval between upper and lower concentration of an analyte in the sample for which it has been showed that the analytical procedure has a suitable level of linearity, accuracy, precision. The obtained range of an analyte is 10 to 50 µg/ml.

**Ruggedness**

Ruggedness of the method was determined by analyzing same sample by different analysts (analyst 1 and 2) at different condition and the respective absorbance were noted and result was indicated as %RSD.

**Robustness**

Robustness of the method was determined by carrying out the analysis at two different wavelength (225 and 232) preparing solution 15 µg/ml.

- **Accuracy (Table No.2)**

**AT 80%**

| Sr no | Sol. | Abs. | Conc.Bef addition [µg/ml] | STD added [µg/ml] | Abs | Conc. after addition[µg/ml] | % Recovery |
|---|---|---|---|---|---|---|---|
| 1 | 10 | 0.710 | 10 | 8 | 0.992 | 18 | 99.28% |
| 2 | 10 | 0.711 | 10 | 8 | 0.995 | 18 | 99.28% |
| 3 | 10 | 0.709 | 10 | 8 | 0.993 | 18 | 99.08% |

| Mean | SD | RSD |
|---|---|---|
| 99.21 | 0.11547 | 0.1163 |

Fig- Recovery Study of Glipizide at 80 %

**AT 100%**

| Sr no | Sol. | Abs. | Conc.Bef addition [µg/ml] | STD added [µg/ml] | Abs | Conc. after addition[µg/ml] | % Recovery |
|---|---|---|---|---|---|---|---|
| 1 | 10 | 0.820 | 10 | 10 | 1.008 | 20 | 99.98% |
| 2 | 10 | 0.821 | 10 | 10 | 1.112 | 20 | 99.26% |
| 3 | 10 | 0.819 | 10 | 10 | 1.114 | 20 | 99.26% |

| Mean | SD | RSD |
|---|---|---|
| 99.5 | 0.41569 | 0.4177 |

Fig- Recovery Study of Glipizide at 100 %

**AT 120%**

| Sr no | Sol. | Abs. | Conc.Bef addition [µg/ml] | STD added [µg/ml] | Abs | Conc. after addition[µg/ml] | % Recovery |
|---|---|---|---|---|---|---|---|
| 1 | 10 | 0.870 | 10 | 12 | 1.112 | 22 | 99.21% |
| 2 | 10 | 0.875 | 10 | 12 | 1.114 | 22 | 99.21% |
| 3 | 10 | 0.876 | 10 | 12 | 1.118 | 22 | 99.12% |

| Mean | SD | RSD |
|---|---|---|
| 99.18 | 0.0519 | 0.05232 |

Fig- Recovery Study of Glipizide at 120 %

**Detection Limit**

1. For 80%

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

$$= \frac{3.3\times 0.1163}{0.03037}$$

$$= 12.65\mu g/ml$$

2. For 100%

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

$$= \frac{3.3 \times 0.4177}{0.0307}$$

$$= 44.89\mu g/ml$$

3. For 120%

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

$$= \frac{3.3\times 0.05232}{0.0307}$$

$$= 5.62\mu g/ml$$

Where

σ = Relative standard deviation of the response.

S = the slope of the calibration curve (of theanalyte).

**Quantitation limit**

Quantitation Limit (LOQ) may be expressed as:

1. For 80%

$$LOQ = \frac{10\,\sigma}{S}$$
$$= \frac{10\times0.1163}{0.0307}$$
$$= 37.88\ \mu g/ml$$

2. For 100%

$$LOQ = \frac{10\,\sigma}{S}$$
$$= \frac{10\times0.4177}{0.0307}$$
$$= 112.89\ \mu g/ml$$

3.For 120%

$$LOQ = \frac{10\,\sigma}{S}$$
$$= \frac{10\times0.4177}{0.0307}$$
$$= 136.05\ \mu g/ml$$

Where

σ = Relative standard deviation of the response.

S = the slope of the calibration curve (of theanalyte).

**Results and Discussion**

Glipizide had high methanol solubility in the current study. Plotting the absorbance against the concentrations of Glipizide in the range of 1–5 µg/ml yielded a linear relationship. The calibration curves for the medicines showed high linearity, as indicated by the correlation value of 0.9977. For Glipizide, the typical linear equation was y = 0.3514x + 0.0562. The results showed that the limits of quantification for 80%, 100%, and 120% were 37.88, 112.89, and 136.05 µg/ml, respectively, while the limits of detection for 80%, 100%, and 120% were 12.65, 44.89, and 5.62 µg/ml.

**CONCLUSIONS**

The study's findings indicated that the suggested approach for determining the amount of glipizide in an active pharmaceutical component was straightforward, accurate, and quick. The technique demonstrated exceptional sensitivity, good linearity, and accuracy.

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