Determination of Glibenclamide By Analytical Spectrophotometry

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ABSTRACT;
Simple and rapid spectrophotometric method was developed and applied to determine Glibenclamide (GB) by zero spectrophotometric method and first derivative spectrophotometric method for determining of (GB) in the presence of Metformin hydrochloride (MET). Zero spectrophotometric (ZS) method was applied for the determination of (GB) at λmax = 300 nm. Linearity range was (4 – 360) µg/mL. Regression analysis showed a good correlation coefficients R² = 0.99993. The limit of detection (LOD) and limit of quantification (LOQ) were to be 0.65 µg/mL and 2.31 µg/mL, respectively. First derivative spectrophotometric (1DS) method was applied for the determination of (GB) in the presence (MET). (GB) was determined at 317 nm (1D317). Linearity ranges were (4 – 240) µg/mL for (GB). Regression analysis showed a good correlation coefficients R² = 0.999914. The limit of detection (LOD) and limit of quantification (LOQ) were to be 0.60 µg/mL and 1.83 µg/mL for (GB).

The proposed zero spectrophotometry method was applied to analysis individual (GB), and the derivative (1D317) method was applied to analysis (GB) individually or combined with (MET) in Syrian trademark drugs.

The proposed method is simple, direct, sensitive and do not require any extraction process. Thus, this method could be readily applicable for the quality control and routine analysis.

KEYWORDS: Glibenclamide (GB), Zero spectrophotometry (ZS), Derivative spectrophotometry (1DS).

INTRODUCTION
Chemically (GB) is 1-[4-[2-[(5-Chloro-2-methoxybenzoyl)amino]ethyl]phenyl]sulphonyl]-3-cyclohexylurea, is oral hypoglycemic drug sulphonyl ureas second generation act by inhibiting ATP-sensitive potassium channels in pancreatic beta cells. This inhibition causes cell membrane depolarization, which cause voltage dependent calcium channels to open, which causes an increase in intracellular calcium in the beta cell, which stimulates insulin release¹-².

The estimation of (GB) from pharmaceutical formulations has been determining by several analytical methods. These include UV spectroscopy methods³-⁹, spectrofluorimetric method¹⁰, reversed-phase high-performance liquid chromatography (RP-HPLC) methods¹¹-¹² and high performance liquid chromatography (HPLC)¹³-¹⁴, high performance thin layer chromatography (HPTLC)¹⁵-¹⁶.

MATERIALS AND METHODS

Apparatus
All spectral measurements were carried out using a Spectro Scan 80 DV, UV/Vis spectrophotometer instrument Ltd (UK), connected to computer, quartz cells 1 cm. Ultrasonic bath Daihan (KORE), and stirrer VelpScientific, (Europe). Sartorius balance, sensitivity 0.01 mg.

Chemical regents
Glibenclamide (GB): C₂₃H₂₈ClN₃O₅S, Mw = 494 g/mol from (China), its purity 99.9%; potassium hydroxide: M_w = 56.11 g/mol from (India), its purity 85 %.

Stock standard preparation
- Stock solution 4.05 × 10⁻³ M of (GB), M_w = 494 g/mol, was prepared by dissolving 40 mg of (GB) in Potassium hydroxide 0.1 M, equivalent to 40.04 mg by taken the purity in consideration in volumetric flask 20 mL. The working standard solutions for zero spectrophotometric method were prepared by appropriate dilutions of stock solution 4.05 × 10⁻³ M in KOH 0.1 M to give concentrations between (4 - 360) µg/mL of (GB). The working standard solutions for derivative spectrophotometric method were...
prepared by appropriate dilutions of stock solution $4.05 \times 10^{-3}$ M in KOH 0.1 M to give concentrations between (4 - 240) µg/mL of (GB).

- Stock solution 0.1 M of potassium hydroxide, (MW = 56.11 g/mol) was prepared by dissolving 5.611 g of KOH equivalent to 6.60 g by taken the purity in consideration in volumetric flask 1000 mL.

**Calibration Curve**

To construct the calibration curve, five standard solutions for each concentration were prepared and the absorbance was measured of each solution five times.

**Sample preparation**

Two products were studied:

- Twenty tablets from Glu-sta Syrian products were weighed and finely powdered and an accurate weight equivalent to 5 mg (GB) was accurately weighed, dissolved in volumetric flask 10 mL in KOH 0.1 M, then 3 mL of the solution was taken to volumetric flask 10 mL and diluted to volume with KOH 0.1 M. 0.7 mL of the last solution was taken to volumetric flask 10 mL then diluted to volume with KOH 0.1 M. The result solution is theoretically equivalent to 10.5 µg/mL for (GB).

- Twenty tablets from GLIBOMET Syrian products were weighed and finely powdered and an accurate weight equivalent to 2.5 mg (GB) was accurately weighed, dissolved in 10 mL volumetric flask in KOH 0.1 M, then 3 mL of the solution was taken to volumetric flask 10 mL and diluted to volume with KOH 0.1 M. 1 mL of the last solution was taken to volumetric flask 10 mL then diluted to volume with KOH 0.1 M, equivalent theoretically to 7.5 µg/mL for (GB).

**RESULTS AND DISCUSSION**

Zero spectrophotometry: Absorption spectra of the standard raw material samples 40 µg/mL (GB) and 15 µg/mL (MET) solutions were recorded within a wavelength range of (225 – 350) nm against the blank 0.1 M of KOH (all the addition constituents without (GB) and (MET)).

On the other hand, derivative spectrophotometry showed more resolution, where the determination of (GB) and (MET) mixture was possible without pretreatment. The first derivative spectrum at zero-crossing point was used to determine (GB) in the presence of (MET) at 317 nm, Fig. 1.

![Fig. 1: First derivative spectra of: a- (GB), b- (MET).](image)

**STABILITY OF STOCK SOLUTION**

Time effect on the stability of standard stock solution of (GB) in KOH 0.1 M was studied in three different concentrations $1.6 \times 10^{-4}$ M, $2.4 \times 10^{-4}$ M and $3.2 \times 10^{-4}$ M. We did not notice any significant changes during the absorption measurement within two months.

**METHOD’S VALIDATION**
The validity and suitability of the proposed method was assessed by linearity (evaluated by regression equation), limit of detection (LOD), limit of quantification (LOQ), accuracy (reported as percent %), precision (reported as RSD %), robustness, and Sandell’s sensitivity.

**Linearity**

We studied the linearity of (GB) standard concentrations at the optimal conditions where we made a series of 10 mL of separated volumetric flasks, each one contains variable concentration of (GB) stock solution $4.05 \times 10^{-3}$ M, and completed to 10 mL with KOH 0.1 M. Finally, we measured the absorbance at 300 nm for each concentration. Fig. 2 presents the (GB) spectra. The range of linearity was obeyed to Beer’s law in concentration (4 - 360) μg/mL and the linearity curve is presented in Fig. 3.

The concentration linearity of (GB) in presence (MET) was in the range (4 – 240) μg/mL at 317 nm by $^{1}D_{317}$, see Figs. 4 & 5.
Limit of Detection (LOD) and Limit of Quantification (LOQ)

In spite of the measurement LOD and LOQ of (GB), five concentrations were analyzed in five replicates. LOD and LOQ for Glibenclamide were calculated by using the following equations:

\[
\text{LOD} = \frac{3.3 \times \text{SD}}{m}, \text{LOQ} = \frac{10 \times \text{SD}}{m}
\]

Where SD, is the standard deviation of y intercepts of regression lines and m is the slope of the calibration curve. The limit of detection (LOD) and limit of quantification (LOQ) were for the first method (Zero spectrophotometry) 0.65 µg/mL and 2.31 µg/mL respectively. and were for second method (Derivative spectrophotometry) 0.60 µg/mL and 1.83 µg/mL respectively.

Accuracy

To determine the precision and accuracy of the proposed method, five replicate determinations were carried out on five different concentrations of standards (GB). The precision and accuracy results are presented in Table 1.

Table 1: Accuracy for determination of Glibenclamide.

| Method | Sample | Theoretical concentration (µg/mL) | Observed concentration (µg/mL) | SD (µg/mL) | Precision RSD (%) | Accuracy (%) |
|--------|--------|---------------------------------|-----------------------------|------------|-----------------|-------------|
| ZS     | Glibenclamide | 4 | 4.04 | 0.03 | 0.74 | 101.00 |
| λ<sub>max</sub>=300 nm |        | 120 | 120.33 | 0.94 | 0.78 | 100.28 |
|        |        | 200 | 200.29 | 0.73 | 0.36 | 100.15 |
|        |        | 280 | 279.86 | 0.85 | 0.30 | 99.95 |
|        |        | 360 | 360.98 | 0.75 | 0.21 | 100.27 |
| DS     | Glibenclamide | 4 | 4.00 | 0.15 | 3.75 | 100.00 |
| λ=317 nm |        | 40 | 39.97 | 0.19 | 0.48 | 99.93 |
|        |        | 80 | 80.04 | 0.24 | 0.30 | 100.05 |
|        |        | 160 | 160.04 | 0.98 | 0.61 | 100.03 |
|        |        | 240 | 239.90 | 0.52 | 0.22 | 99.96 |

Precision

In order to demonstrate the precision of the proposed method, intra-day and inter-day variability studies were performed at three different concentrations (20, 40, and 80) µg/mL for of standards (GB) at the same day in two hour’s time interval and also at three different days. Method efficiency was tested in terms of RSD % for
both intra-day and inter-day precisions. The precision was ascertained by carrying out five replicates of standards (GB) under study and the mean was calculated. The results are showed in Tables 2 & 3. The RSD% results were not more than 1.51% and 1.85% for of standards (GB) using (ZS) method and of standards (GB) using (1DS) method respectively, where the method is considered very precise.

Table 2: Intra-day precision for determination of Glibenclamide.

| Method | Sample       | Concentration µg/mL | Found concentration µg/mL | *Time I Precision RSD% | *Time II Precision RSD% | *Time III Precision RSD% |
|--------|--------------|---------------------|---------------------------|------------------------|-------------------------|--------------------------|
| ZS     | Glibenclamide| 20                  | 20.17                     | 0.61                   | 20.19                   | 1.51                     |
|        |              | 40                  | 40.37                     | 0.75                   | 40.78                   | 0.83                     |
|        |              | 80                  | 80.21                     | 0.33                   | 80.40                   | 0.56                     |
| 1DS    | Glibenclamide| 20                  | 20.52                     | 0.91                   | 20.31                   | 1.19                     |
|        |              | 40                  | 40.11                     | 0.60                   | 40.25                   | 0.97                     |
|        |              | 80                  | 79.84                     | 0.65                   | 79.42                   | 1.34                     |

* n = 5

Table 3: Inter-day precision for determination of Glibenclamide.

| Method | Sample       | Concentration µg/mL | Found concentration µg/mL | *Time I Precision RSD% | *Time II Precision RSD% | *Time III Precision RSD% |
|--------|--------------|---------------------|---------------------------|------------------------|-------------------------|--------------------------|
| ZS     | Glibenclamide| 20                  | 20.17                     | 0.61                   | 20.32                   | 1.35                     |
|        |              | 40                  | 40.37                     | 0.75                   | 39.96                   | 0.37                     |
|        |              | 80                  | 80.21                     | 0.33                   | 80.83                   | 0.99                     |
| 1DS    | Glibenclamide| 20                  | 20.52                     | 0.91                   | 20.45                   | 1.49                     |
|        |              | 40                  | 40.11                     | 0.60                   | 40.25                   | 1.29                     |
|        |              | 80                  | 79.84                     | 0.65                   | 80.18                   | 1.15                     |

* n = 5

Robustness

The robustness of an analytical procedure is a measure of its capacity to maintain unaffected results by a very small variation of some parameters and provides an indication of its reliability during normal usage. The studied variables parameters were slit, scan speed and the wavelength which performed at three different concentrations (40, 60 and 80) µg/mL for (GB). The results in Table 4 showed no significant difference.

Table 4: Robustness test.

| Method | parameter | Deviation (µg/mL) | SD (µg/mL) | Per RSD% | SD (µg/mL) | RSD% | Per | SD (µg/mL) | RSD% | Per |
|--------|-----------|-------------------|------------|----------|------------|------|-----|------------|------|-----|
| ZS     | slit rang (2 nm) | 40.19 | 0.38 | 0.95 | 100.48 | 60.03 | 0.52 | 0.87 | 100.05 | 80.45 | 0.28 |
|        | slit rang (1 nm) | 40.29 | 0.43 | 1.07 | 100.73 | 59.96 | 0.69 | 1.15 | 99.93 | 80.39 | 0.25 |
|        | Scan speed Fast | 40.19 | 0.38 | 0.95 | 100.48 | 60.03 | 0.52 | 0.87 | 100.05 | 80.45 | 0.28 |
|        | Scan speed Slow | 39.98 | 0.26 | 0.65 | 99.95 | 60.10 | 0.97 | 1.61 | 100.17 | 80.22 | 0.49 |

* n = 5
\*n=5.

**Sensitivity Sandell’s and molar absorptivity**

Sensitivity of the proposed method for (GB) was determined by calculating Sandell's sensitivity (SS), it was to be 0.378 µg/cm\(^2\). The mean molar absorptivity \(\epsilon\) was found equal to 2613.8 L/mol.cm.

**RECOVERY**

The recovery was studied by three addition standards (80 %, 100 %, and 120 %) for every product.

Table 5 presents the recoveries results for the two Syrian products (Glu-staand GLIBOMET).

### Table 5: Recoveries of Glibenclamide in Glu-sta and GLIBOMET products.

| Method | Products | Pharmaceutical dosage | Sample \(\mu g/mL\) | Added \(\mu g/mL\) | Total Found \(\mu g/mL\) | Recovery Average\% | SD \(\mu g/mL\) | RSD\% | Recovery Average\% |
|--------|----------|------------------------|---------------------|------------------|-----------------------------|---------------------|-----------------|--------|---------------------|
| **ZS** | Glu-sta  | Glibenclamide 5 mg/tab. | 10.45               | 8.36             | 18.79                       | 99.76               | 1.14            | 1.14               | 100.16                         |
|        |          |                        | 10.45               | 10.45            | 20.85                       | 99.52               | 2.52            | 2.53               |                                  |
|        |          |                        | 10.45               | 12.54            | 23.14                       | 101.19              | 2.22            | 2.19               |                                  |
| **1DS**| Glu-sta  | Glibenclamide 5 mg/tab. | 10.42               | 8.34             | 18.81                       | 100.60              | 2.68            | 2.66               | 100.24                         |
|        |          |                        | 10.42               | 10.42            | 20.86                       | 100.19              | 1.77            | 1.77               |                                  |
|        |          |                        | 10.42               | 12.50            | 22.91                       | 99.92               | 2.30            | 2.30               |                                  |
| **ZS** | GLIBOMET | Glibenclamide 2.5 mg/tab. | 7.46                | 5.97             | 13.49                       | 101.00              | 2.43            | 2.41               | 100.41                         |
|        |          |                        | 7.46                | 7.46             | 14.87                       | 99.33               | 2.46            | 2.48               |                                  |
|        |          |                        | 7.46                | 8.95             | 16.49                       | 100.89              | 2.76            | 2.74               |                                  |
| **1DS**| GLIBOMET | Glibenclamide 2.5 mg/tab. | 7.48                | 5.98             | 13.41                       | 99.16               | 2.42            | 2.44               | 99.93                          |
|        |          |                        | 7.48                | 7.48             | 14.99                       | 100.40              | 2.56            | 2.55               |                                  |
|        |          |                        | 7.48                | 8.98             | 16.48                       | 100.22              | 2.45            | 2.44               |                                  |

\(\overline{x}\) Means five separated determinations.
APPLICATION

Estimation of Glibenclamide in Glu-sta and GLIBOMET products

The developed method was applied for quantitative determination and identification (GB) in two Syrian pharmaceutical products (Glu-sta and GLIBOMET), for three different batches for each preparation. The samples were prepared as described in the section of samples preparation and analyzed. Quantitative analysis was done by using calibration curve. The obtained results are summarized in Table 6. In general, the concentrations of the detected (GB).

USP legislation\(^7\) indicate that the tablets must contain not less than 90.00 % and not more than 110.00 % of labeled amount. So, the obtained results are conformed to USP legislation\(^7\). The relative standard deviations RSD % (n = 5) of the quantitative results were in the range of 1.00 – 2.22% for Glu-sta and 1.61 – 1.98% for GLIBOMET, Table 6.

| Method | Product | Glu-sta 5 mg/tab. | GLIBOMET 2.5 mg/tab. |
|--------|---------|------------------|---------------------|
|        |         | Concentration mg/tab. |              |              |
| ZS     |         | 4.98  | 5.07  | 4.96  | 2.49  | 2.52  | 2.55  |
|        |         | Range mg/tab. | 4.96 – 5.07 | 2.49 – 2.55 |
|        |         | SD mg/tab. | 0.05  | 0.11  | 0.11  | 0.04  | 0.05  | 0.05  |
|        |         | RSD %    | 1.00  | 2.17  | 2.22  | 1.61  | 1.98  | 1.96  |
|        |         | Range RSD % | 1.00 – 2.22 | 1.61 – 1.98 |
|        |         | Per %    | 99.6  | 101.4 | 99.2  | 99.6  | 100.8 | 102.0 |
|        |         | Range Per % | 99.2 – 101.4 | 99.6 – 102.0 |
| DS     |         | Concentration mg/tab | 4.96  | 5.02  | 4.84  | 2.53  | 2.49  | 2.57  |
|        |         | Range mg/tab | 4.84 – 5.02 | 2.49 – 2.57 |
|        |         | SD mg/ tab | 0.18  | 0.15  | 0.19  | 0.03  | 0.06  | 0.06  |
|        |         | RSD %    | 3.63  | 2.99  | 3.93  | 1.19  | 2.41  | 2.33  |
|        |         | Range RSD % | 2.99 – 3.93 | 1.19 – 2.41 |
|        |         | Per %    | 99.2  | 100.4 | 96.8  | 101.2 | 99.6  | 102.8 |
|        |         | Range Per % | 96.8 – 100.4 | 99.6 – 102.8 |

Mean for five replicates. \(\bar{x}\)

CONCLUSION

We developed a new method which is suitable for the identification and quantification of Glibenclamide in raw material and Syrian tablets formulation. A good percentage of recovery shows that the method can be successfully used in pharmaceutical quality control and routine analyses. The proposed method is simple, sensitive, rapid, specific, a little cost. It could be applied for quality control of Glibenclamide in pharmaceutical factories. The levels of Glibenclamide compounds in the analyzed preparations were found to be within the permissible limits set by the USP legislation\(^7\).
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