Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol-13: Issue 8
Open Access

# RP-HPLC Method Development And Validation Of Glipizide And Glibenclamide In Bulk And Pharmaceutical Dosage Form

**Ms. Sharmila Pratap Gandhi[1], Dr. Sonia Singh[2]**

1. Ph.D. Scholar, Department of Pharmaceutics, Sinhgad College of Pharmacy, Vadgaon (BK), Pune.
2. Principal, Alard college of Pharmacy, Pune.

**Corresponding Author**

Ms. Sharmila Pratap Gandhi, Ph.D. Scholar, Department of Pharmaceutics, Sinhgad College of Pharmacy, Vadgaon (BK), Pune.

Email Id: Sharmila.shahsvpm@gmail.com

Cite this paper as: Ms. Sharmila Pratap Gandhi, Dr. Sonia Singh (2024). RP-HPLC Method Development And Validation Of Glipizide And Glibenclamide In Bulk And Pharmaceutical Dosage Form. Frontiers in Health Informatics, Vol. 13, No.8, 7421-7439

## Abstract

A precise and robust method was developed for estimation of Glipizide and Glibenclamide in bulk and formulations by RP-HPLC technique. The Method used Agilent 1260 Infinity II model HPLC with DAD detector and Agilent Zorbax Bonus RP Column with dimension 250 x 4.6 mm, 5 µm. The Mobile phase combination used was 0.1% Trifluoroacetic acid (TFA) and Methanol (30:70), flow rate at 1.0 ml/min and wavelength at 211 nm with run time of 15 minutes. The retention time of Glipizide and Glibenclamide peaks were at 4.91 and 9.45 minutes, respectively. The method was validated as per ICH guidelines. The instrument precision for Glipizide and Glibenclamide had a %RSD of 0.08% and 0.13%, respectively. Method was linear and accurate for concentration range 40-60µg/ml for both Glipizide and Glibenclamide with regression coefficient of 0.9994 and 0.9997, respectively. % RSD for accuracy for Glipizide at 80%, 100% and 120% was found to be 0.30%, 0.05% and 0.07%, respectively; and for Glibenclamide at 80%, 100% and 120% was found to be 0.56%, 0.16% and 0.11% respectively.

## 1. Introduction

Glipizide and Glibenclamide are used to treat Diabetes Mellitus. Glipizide is a sulfonylurea of the second generation and has been given the green light by the FDA for use in the treatment of individuals who suffer from type 2 diabetes. It is meant to be used in conjunction with a healthy diet and regular exercise. It can be used in conjunction with the biguanide metformin to achieve the desired HbA1c in patients who have not been able to achieve acceptable metabolic control in three months despite adhering to their prescribed diet, exercise, and medication regimens. It is possible for it to be used as a monotherapy in certain circumstances, such as when there is an intolerance to metformin or when its usage is contraindicated. Glipizide and the other sulfonylureas are common alternatives for doctors since they are effective in the management of type 2 diabetes, come at a lower price point, and are readily available.[1]

Glibenclamide, which is also known as glyburide, is a sulfonylurea of the second generation that is widely used for the treatment of type 2 diabetes mellitus as well as gestational diabetes [2]. An exhaustive review of the relevant scientific literature indicated that numerous analytical methods have been described for the analysis of Glibenclamide either on its own or in combination with other hypoglycemic agents in pure pharmaceuticals, marketed tablets, and biological fluids (such as plasma, serum, and urine). These methods can be applied to determine if Glibenclamide is present alone or in conjunction with the other hypoglycemic agents.
The chemical name (IUPAC) of Glipizide is N-[2-[4-(cyclohexylcarbamoylsulfamoyl)phenyl] ethyl]-5-methylpyrazine-2-carboxamide (Figure 1).

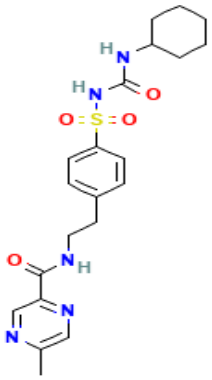


**Figure 1: Chemical Structure of Glipizide[3]**

The chemical name (IUPAC) of Glipizide is 5-chloro-N-[2-[4-(cyclohexylcarbamoylsulfamoyl)phenyl]ethyl]-2-methoxybenzamide (Figure 2).

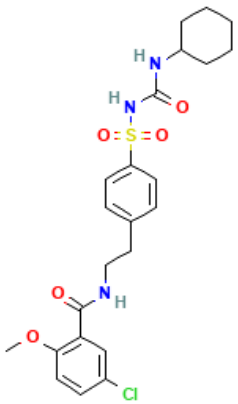


**Figure 2: Chemical Structure of Glibenclamide [4]**

According to the literature review [5-15], there was few Liquid Chromatography analysis for Simultaneous estimation of GLIPIZIDE & GLIBENCLAMIDE in Combination pharmaceutical dosage form. So, current study was planned for development and validation of method developed for Glipizide and Glibenclamide.

**Table No. 1: Quality Target Profile for HPLC Method development**

| Parameter | Limits |
|---|---|
| Theoretical Plates | Not less than 2000 |
| Asymmetry | Not More than 2.0 (Fairly at 1.0) |
| Tailing Factor | Not More than 2.0 (Fairly at 1.0) |
| Run time | Not More than 20 minutes |
| Resolution | Not Less than 2.0 |

## 2. Material and Method

### 2.1. Chemicals and Reagents

Aadhaar Life Sciences Pvt. Ltd. provided a complimentary sample of Glipizide and Glibenclamide. Methanol was purchased from Qualigens in India and was of HPLC grade. Trifluoroacetic acid was purchased from Merck in India and was of AR grade. Internal Milli-Q system provided water. All weighing was done using calibrated NABL scales. Samples were produced in Type A glassware and using the analytical balance.

### 2.2. Instrumentation

Agilent 1260 Infinity II with a DAD detector and quaternary pump was the tool utilized for development and validation. Agilent's Open lab Ezchrom software was employed. The labman ultrasonicator and the Aczet analytical balance were used for wet chemistry.

### 2.3. HPLC Method Development

2.3.1. The table 2 and 3 describes trials done during the development phase with the results and observations.

**Table No. 2. Method development trials**

| Trial No. | Mobile Phase | Mobile phase Ratio | Diluent | Column | Wavelength |
|---|---|---|---|---|---|
| 1 | 0.1% TFA-Methanol | 50-50 | 0.1% TFA-Methanol (50-50) | Agilent Zorbax Bonus RP (250 x 4.6 mm, 5µ) | 250 |
| 2 | 0.1% TFA-Methanol | 40-60 | 0.1% TFA-Methanol (50-50) | Agilent Zorbax Bonus RP (250 x 4.6 mm, 5µ) | 250 |
| 3 | 0.1% TFA-Methanol | 30-70 | 0.1% TFA-Methanol (50-50) | Agilent Zorbax Bonus RP (250 x 4.6 mm, 5µ)) | 210 |

**Table No. 3: Results of Method Development**

| Trial No. | Glipizide | | | | Glibenclamide | | | |
|---|---|---|---|---|---|---|---|---|
| | RT | TP | Asymmetry | Resolution | RT | TP | Asymmetry | Resolution |
| 1 | No Peak Observed | | | | No peak Observed | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 2 | 10.05 | 6734 | 1.03 | 0.00 | 14.01 | 14524 | 1.04 | 8.31 |
| **3** | **5.53** | **6889** | **1.07** | **0.00** | **11.92** | **7254** | **1.03** | **15.45** |

For all the above trials, wavelength was kept constant at 211 nm, as this was predetermined using HPLC DAD detector. Diluent was kept constant as 50-50 0.1%TFA - Methanol for all trails. Column used for all trails was Agilent Zorbax Bonus RP (250 x 4.6 mm, 5 micron). Based in the predetermined quality target profile for development work, the condition for trial 3 was finalized and individual Standard were ran to confirm the retention times. The chromatogram of method development were shown in figure 3.

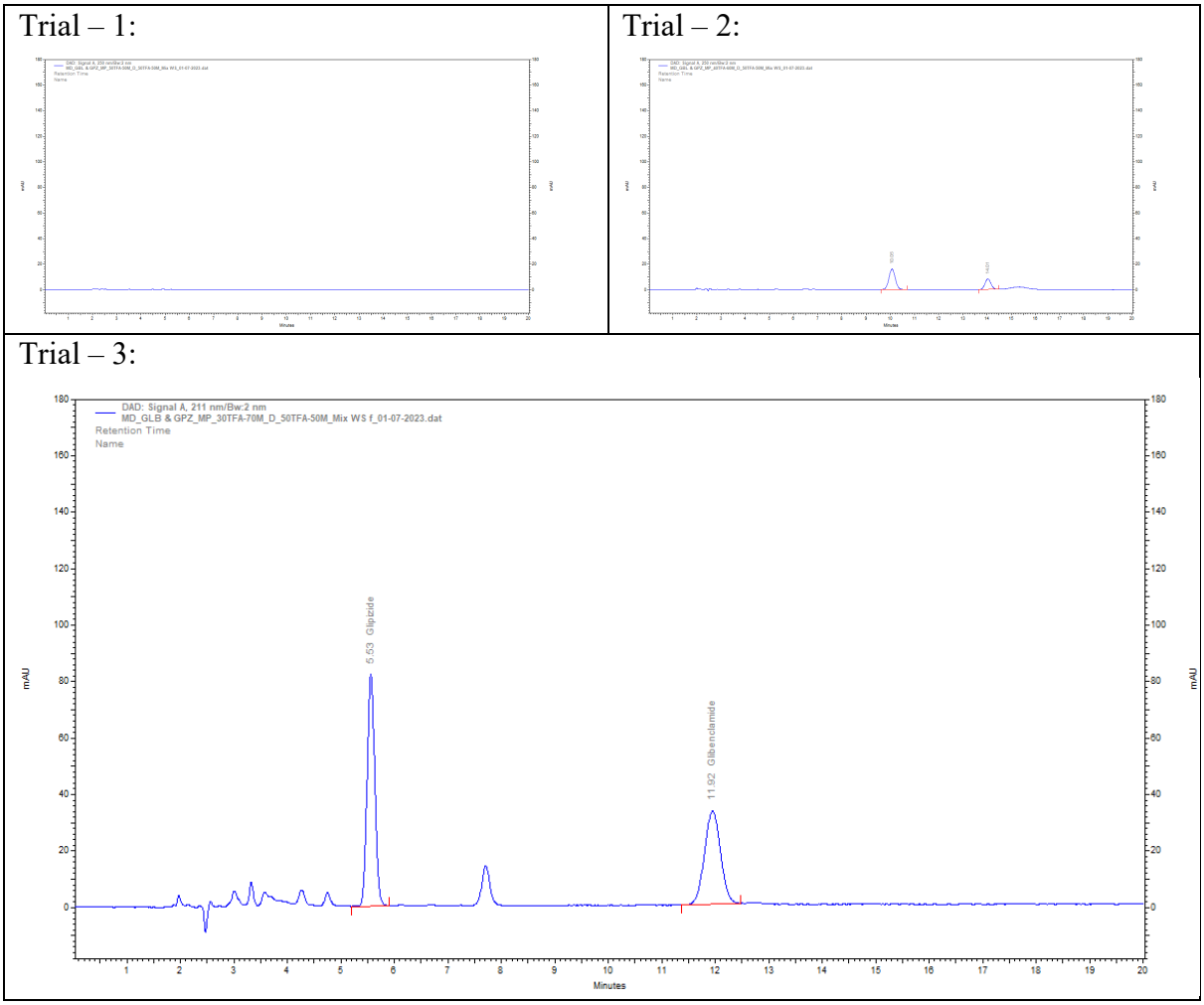



**Figure 3: Method Development Trials**

### 2.3.2. Final Chromatographic Conditions:

**Table No. 4: Final Chromatographic Condition**

| Parameter | Condition |
|---|---|
| HPLC Instrument | Agilent 1260 Infinity II |
| Column | Agilent Zorbax Bonus RP, 5µ, 100A, 250 x 4.60 mm |
| Wavelength | 211 nm |

| | |
|---|---|
| Mobile Phase | 0.1% Trifluoroacetic acid : Methanol (30:70) v/v |
| Diluent | 0.1% Trifluoroacetic acid : Methanol (50:50) v/v |
| Run time | 15 minutes |
| Injection Volume | 10 micro liters |
| Flow Rate | 1.0 ml/min |
| Column oven Temperature | 30°C (± 2°C allowed by Robustness) |

### 2.3.3. Preparation of Mobile Phase

**Preparation of 0.1% Trifluoroacetic acid**

**Take 800 mL of water using graduated cylinder. Pipette out 1 ml of Trifluoroacetic acid and add this to measured water, mix well then adjust the volume to 1000 ml using water.**

**Mobile Phase: 30%- 0.1% TFA : 70% Methanol**

**Mix separately measured 300 mL of 0.1% Trifluoroacetic acid and 700 mL of Methanol into a suitable container. Filter the mobile phase through 0.45 µm nylon membrane filter. Briefly sonicate to degas.**

### 2.3.4. Preparation of Diluent

Mix separately measured 500 mL of 0.1% TFA with 500 mL of Methanol into a suitable container and mix well. Mixture is to be filtered through 0.45 µm nylon membrane filter. Briefly sonicate to degas.

### 2.3.5. Preparation of Standard Solution

**A. Working Standard:**

1. Glipizide Stock Solution-I (GSS-I):
   Prepare a Glipizide Stock Solution (GSS-I) by adding 5 mg of Glipizide in 10 ml volumetric flask & add 5 ml Methanol, mix for 2 minutes and make the volume to 10 ml with Methanol. (Conc. of Glipizide = 500 µg/ml).
2. Glibenclamide Stock Solution-I ( GBSS-II):
   Prepare a Glibenclamide Stock Solution (GBSS-II) by adding 5 mg of Glibenclamide in 10 ml volumetric flask & add 5 ml Methanol, mix for 2 minutes and make the volume to 10 ml with Methanol. (Conc. of Glibenclamide = 500 µg/ml).
3. Add 1.0 ml of GSS-I and 1.0 ml of GBSS-II in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Glipizide = 50 µg/ml, Glibenclamide = 50 µg/ml).

**B. Preparation of Sample for Assay**

As there was no Fixed Dose Combination available in the market. Physical mixture of both the tablets was prepared and used for sample preparation. Weigh 10 tablets of each

drug and calculate average weight of 1 tablet, transfer tablets into mortar and pestle and crush them. Weigh powder equivalent to 5 mg of Glipizide and 5 mg of Glibenclamide and transfer to 10 ml volumetric flask & add 5-7 ml diluent, mix for 5 minutes and make the volume to 100 ml with diluent. (Conc. of Glipizide =500 µg/ml and Glibenclamide = 500 µg/ml). Then add 1.0 ml of above stock solution in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent Conc. of Glipizide = 50 µg/ml and Glibenclamide = 50 µg/ml).

### 2.4. Method validation

#### 2.4.1. Specificity

Individual injections of Glipizide and Glibenclamide were prepared of 50 µg/ml and 50 µg/ml, respectively and peaks were identified from Retention Time. Blank was injected to ensure there is no blank peak interfering with the main analyte peaks.

#### 2.4.2. System Suitability

Using a series of tests, the suitability and performance of the system were examined. Theoretical Plate count, tailing factor, and resolution are all found to be within allowed ranges for the ICH guideline system.

#### 2.4.3. Accuracy

To determine the accuracy of a technique, one must examine how closely its test findings correspond to the actual value. In the recovery studies, three distinct concentration levers were evaluated. At each level, three replicate injections were performed and the amount of drug present, the percentage of recovery, and the related standard deviation were calculated.

#### 2.4.4. Repeatability

Analytical precision is determined by the degree of concordance between individual test results. Multiple samples of a uniform sample were examined. A single sample was prepared as described and 6 injections were made from same sample and checked for system suitability. Instrument precision was performed as Instrument precision (how good the instrument preform back to back replicate injection of same concentration).

#### 2.4.5. Linearity

Methodological linearity is the capacity of an analytical method to yield results proportionate to analyte concentrations within a given range. There were five sets of standard solutions used to determine linearity. On the calibration curve, the peak area against concentration of the standard solution was plotted, and the regression equation was developed. The least-squares method was utilized to determine the slope, intercept, and correlation coefficient.

#### 2.4.6. LOD and LOQ

The LOD and LOQ are denoting ability of the method to detect and quantify smallest amount of analyte, respectively. The LOD and LOQ were calculated by using standard deviation and slope of regression line by using following equations.

### 2.4.7. Robustness

The Robustness was performed changing the column temperature by ± 2˚C and Wavelength by ± 2 nm.

**Table No. 5: Robustness Trials**

| Condition | Increased | Normal | Decreased |
|---|---|---|---|
| **Column Oven Temperature** | 32˚C | 30˚C | 28˚C |
| **Wavelength** | 213 nm | 211 nm | 209 nm |

### 2.4.8. Inter-day & Intraday Precision:

The prepared working standard was analyzed in morning and at evening and % RSD was calculated to identify the stability of solution for intraday precision. The same solution was injected on second day and compared with morning results of intraday precision and % RSD was calculated.

### 2.4.9. Forced degradation

i. **Acid Hydrolysis:** Weigh accurately 5 mg Glipizide and 10 mg of Glibenclamide in 10 ml Volumetric Flask and add 1 ml 5N HCl and store it at room temperature for 60 min. Later dilute this mixture with diluent up to the mark. Further pipette out 1 ml from this solution into in 10 ml Volumetric Flask and make up the volume with diluent.

ii. **Base Hydrolysis:** Weigh accurately 5 mg Glipizide and 10 mg of Glibenclamide in 10 ml Volumetric Flask and add 1 ml 5 N NaOH and store it at room temperature for 60 min. Later dilute this mixture with diluent upto the mark. Further pipette out 1 ml from this solution into in 10 ml Volumetric Flask and make up the volume with diluent.

iii. **Oxidation:** Weigh accurately 5 mg Glipizide and 10 mg of Glibenclamide in 10 ml Volumetric Flask and add 1 ml 30% Hydrogen Peroxide and store it at room temperature for 60 min. Later dilute this mixture with diluent upto the mark. Further pipette out 1 ml from this solution into in 10 ml Volumetric Flask and make up the volume with diluent.

iv. **Oxidation with Heat :** Weigh accurately 5 mg Glipizide and 10 mg of Glibenclamide in 10 ml Volumetric Flask and add 1 ml 30% Hydrogen Peroxide and Heated at 60°C for 60 min. Later dilute this mixture with diluent upto the mark. Further pipette out 1 ml from this solution into in 10 ml Volumetric Flask and make up the volume with diluent.

v. **Dry Heat:** Weigh accurately 5 mg Glipizide and 10mg of Glibenclamide in 10 ml Volumetric Flask and store it at 105˚C for 4 hrs. Later dilute this sample with diluent up to the mark. Further pipette out 1 ml from this solution into in 10 ml Volumetric Flask and make up the volume with diluent.

vi. **Photolysis:** Weigh accurately 5 mg Glipizide and 10 mg of Glibenclamide in 10 ml Volumetric Flask and store it at 254 nm for 4 hrs. Later dilute this sample with diluent up to the mark. Further pipette out 1 ml from this solution into in 10 ml Volumetric Flask and make up the volume with diluent.

## 3. Results and Discussion

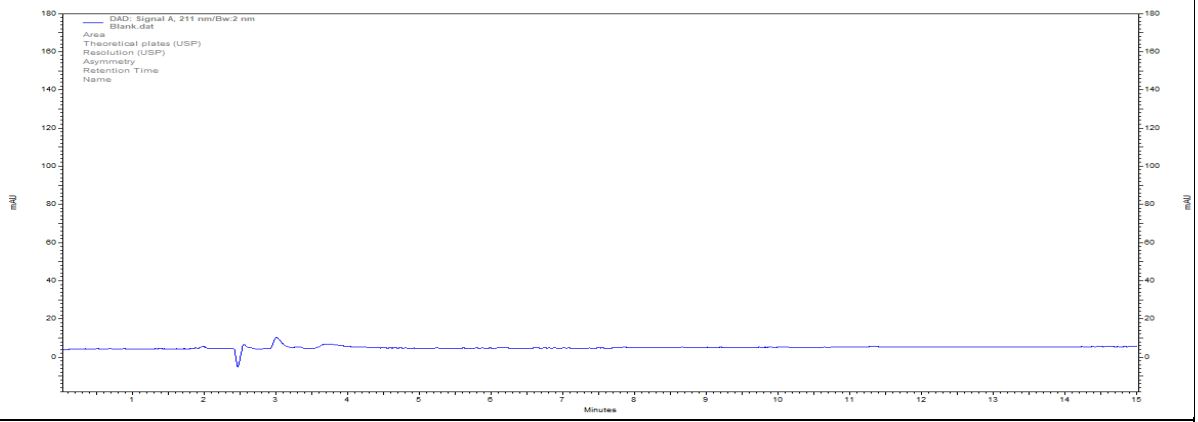
### 3.1. Specificity

Specificity was performed to check if there was any interaction between the peaks from blank or the APIs.

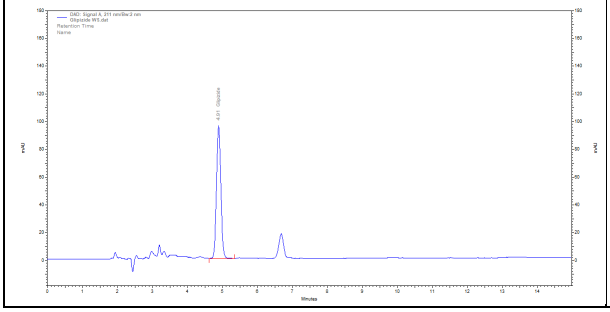
**Table no. 6: Specificity and ID of GLIPIZIDE & GLIBENCLAMIDE**

| Sample ID | Glipizide | Glibenclamide |
|---|---|---|
| | RT | RT |
| **Blank** | - | - |
| **Glipizide WS** | 4.91 | - |
| **Glibenclamide** | - | 9.45 |
| **MIX WS** | 4.91 | 9.45 |
| **Drug Product** | 4.91 | 9.45 |

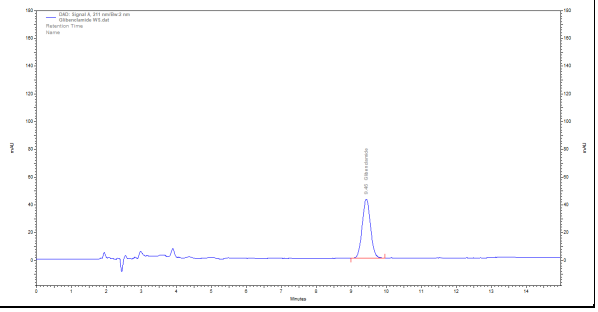
a. Diluent




b. Glipizide WS



c. Glibenclamide WS



d. Mixed Working Standard of GLIPIZIDE & GLIBENCLAMIDE

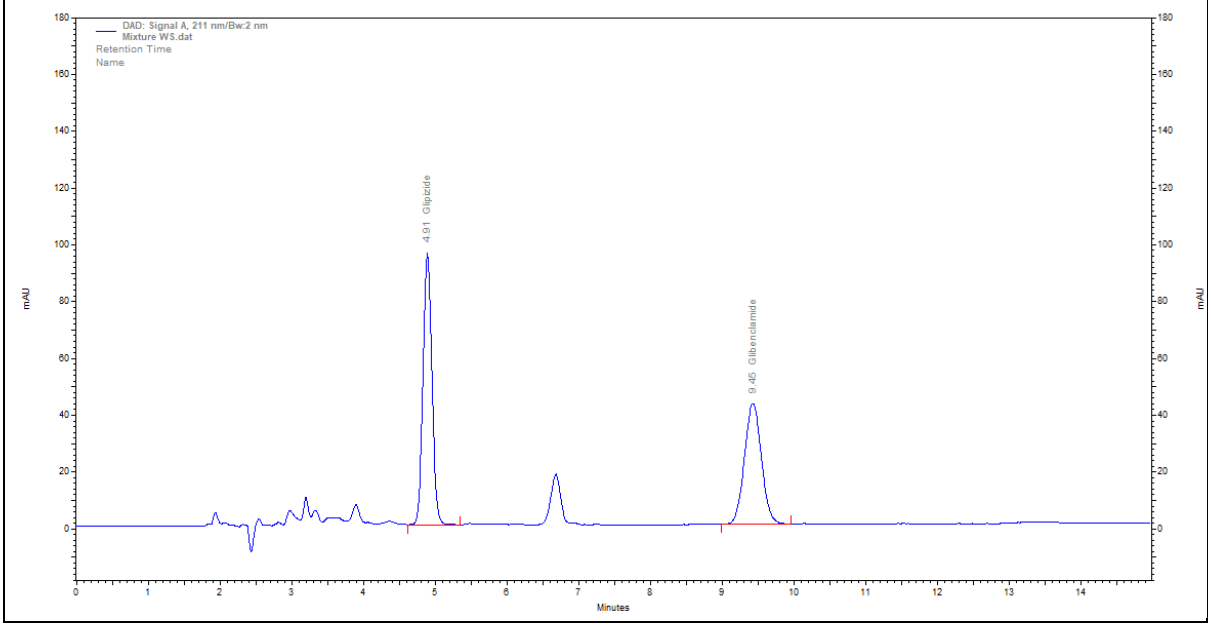



**Figure No. 4: Chromatogram ID. a] Diluent, b] Glipizide, c] Glibenclamide, d] Mixture Working Standard of GLIPIZIDE & GLIBENCLAMIDE.**

### 3.2. Instrument Precision and System suitability

The HPLC Instrument was tested for its suitability to perform the validation. Based on the limits mentioned in table 1, the equipment was found to be suitable for continuing the validations. Instrument precisions of both the drugs were performed after system suitability and the reported data in below shows the relative standard deviation for Instrument precision of GLIPIZIDE & GLIBENCLAMIDE are 0.08% and 0.13% respectively. This %RSD shows the method is very much precise with respect to multiple sample preparation for same concentration. The data is shown in table 7-9.

Table 7: System suitability for Glipizide

| Sample ID | Glipizide | | | |
|---|---|---|---|---|
| | RT | TP | Asymmetry | Resolution |
| 100% Rep 1 | 4.91 | 1.07 | 7274 | 0.00 |
| 100% Rep 2 | 4.91 | 1.05 | 7454 | 0.00 |
| 100% Rep 3 | 4.91 | 1.02 | 7641 | 0.00 |
| 100% Rep 4 | 4.91 | 1.07 | 7265 | 0.00 |
| 100% Rep 5 | 4.91 | 1.06 | 7584 | 0.00 |
| 100% Rep 6 | 4.91 | 1.05 | 7411 | 0.00 |
| Average | 4.91 | | | |
| STDEV | 0.00 | | | |
| RSD | 0.00 | | | |

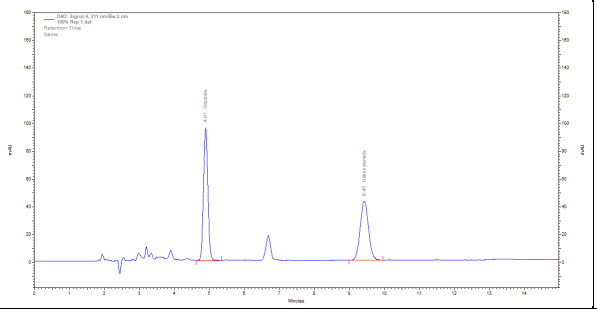
Table 8: System suitability for GLIBENCLAMIDE

| Sample ID | Glibenclamide | | | |
|---|---|---|---|---|
| | RT | TP | Asymmetry | Resolution |
| 100% Rep 1 | 9.45 | 1.04 | 7354 | 13.54 |
| 100% Rep 2 | 9.45 | 1.03 | 7541 | 13.54 |
| 100% Rep 3 | 9.45 | 1.04 | 7365 | 13.54 |
| 100% Rep 4 | 9.45 | 1.06 | 7555 | 13.54 |
| 100% Rep 5 | 9.45 | 1.04 | 7854 | 13.54 |
| 100% Rep 6 | 9.45 | 1.05 | 7454 | 13.54 |
| Average | 9.45 | | | |
| STDEV | 0 | | | |
| RSD | 0.00 | | | |

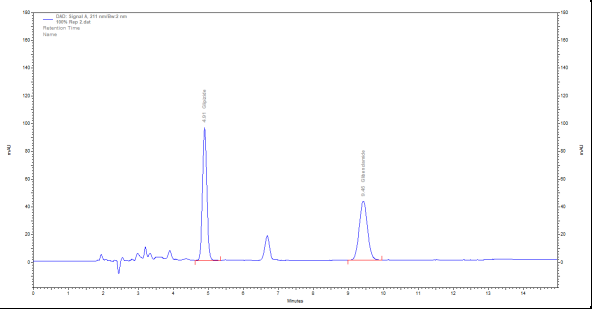
Table 9: Instrument Precision for GLIPIZIDE & GLIBENCLAMIDE

| Repeatability | | |
|---|---|---|
| Sample ID | GLIPIZIDE Area | GLIBENCLAMIDE Area |
| 100% Rep 1 | 1745407 | 1472655 |
| 100% Rep 2 | 1745102 | 1475455 |
| 100% Rep 3 | 1746841 | 1470845 |
| 100% Rep 4 | 1748541 | 1472546 |
| 100% Rep 5 | 1744585 | 1470236 |
| 100% Rep 6 | 1745874 | 1473654 |
| Average | 1746058 | 1472565 |
| STDEV | 1436.6419 | 1893.6274 |
| % RSD | 0.08 | 0.13 |

100% Rep 1



100% Rep 2



100% Rep 3

100% Rep 4

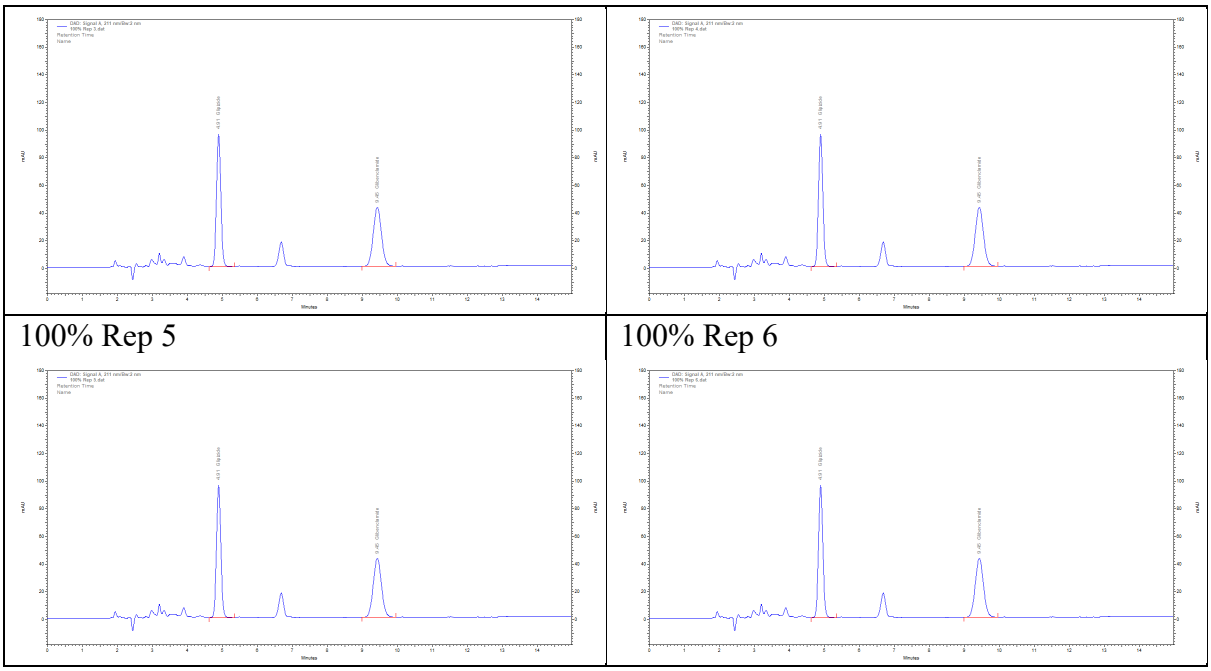


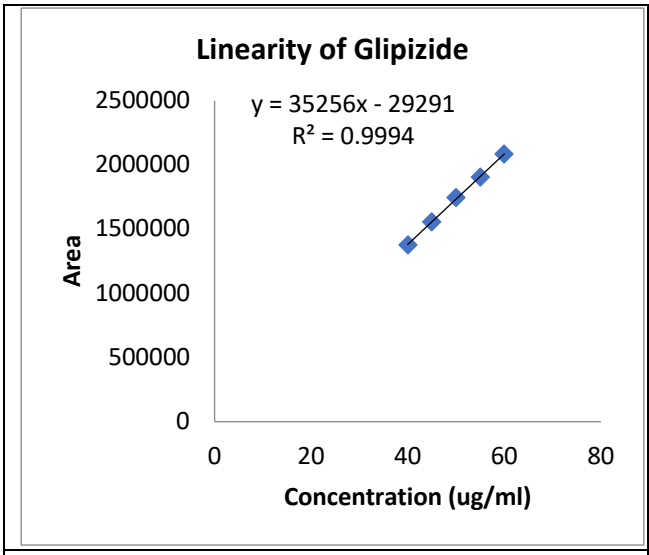

Figure No. 5: Instrument Precision GLIPIZIDE & GLIBENCLAMIDE

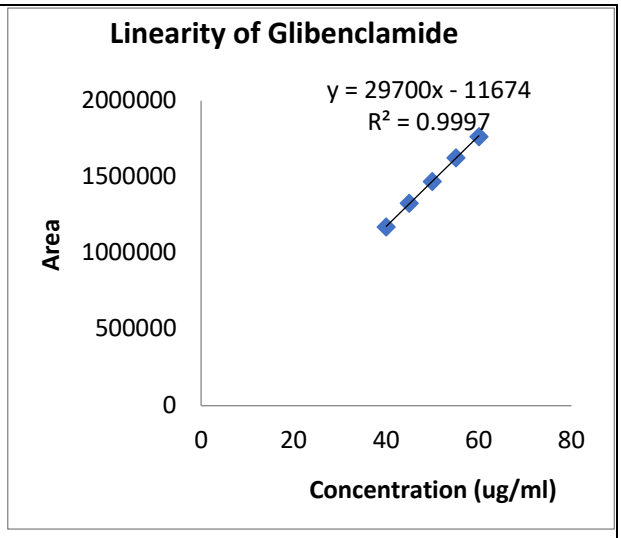
### 3.3. Linearity for GLIPIZIDE and GLIBENCLAMIDE

Linearity was performed at different levels. The graph plotted between peak area and concentration showed linearity with correlation coefficient as shown in table below. The linearity data in shown in table 10 and graph in figure 6.

Table No. 10: Linearity data of GLIPIZIDE & GLIBENCLAMIDE

| Glipizide | | | Glibenclamide | | |
|---|---|---|---|---|---|
| **% Level** | **Conc (ug/ml)** | **Area** | **% Level** | **Conc (ug/ml)** | **Area** |
| 80 | 40 | 1377417 | 80 | 40 | 1172396 |
| 90 | 45 | 1555315 | 90 | 45 | 1328989 |
| 100 | 50 | 1745407 | 100 | 50 | 1472655 |
| 110 | 55 | 1905697 | 110 | 55 | 1626428 |
| 120 | 60 | 2083617 | 120 | 60 | 1766178 |
| $R^2$= 0.9994 | | | $R^2$= 0.9997 | | |



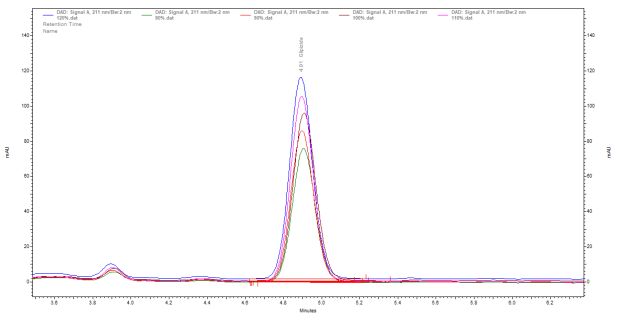



Figure 6.a

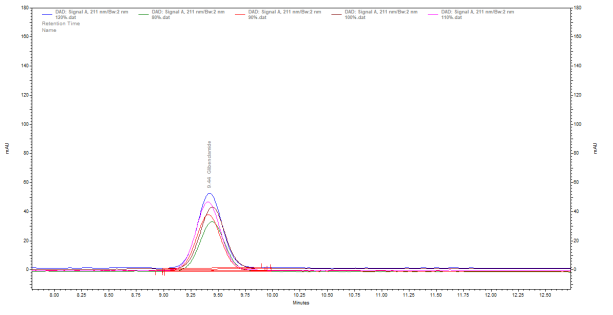


Figure 6.b

Figure No. 6.: Linearity Plot for GLIPIZIDE & GLIBENCLAMIDE, Fig. 6.a) Linearity graph of Glipizide, 6.b) Linearity graph of Glibenclamide.

### 3.4. LOD and LOQ for GLIPIZIDE & GLIBENCLAMIDE

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined for GLIPIZIDE and GLIBENCLAMIDE. The results of analysis are shown in table 11.

Table No. 12. LOD and LOQ for GLIPIZIDE & GLIBENCLAMIDE

| Name | LOD (µg/mL) | LOQ (µg/mL) |
|---|---|---|
| **Glipizide** | 2.32 | 7.03 |
| **Glibenclamide** | 1.73 | 5.25 |

The LOD and LOQ were significantly low, implying the method to be very efficient in determining low concentration of drug. This value of LOD and LOQ can be used during cleaning validation in industry which can help companies know if the manufactured vessel or equipment is free from APIs stains.

### 3.5. Accuracy

Accuracy for GLIPIZIDE was performed in triplicates and it was observed that the method was

accurate for the range 80%, 100% and 120%. The relative standard deviation for 80%, 100% and 120% were 0.30%, 0.05% and 0.07% respectively. The accuracy determined the methods ability to analyses different concentration of drug in solution accurately. The accuracy data is shown in table 12.

Table No.12: Accuracy data for Glipizide

| Sample ID | Reps | Spiked Conc (ug/ml) | Area | Amount Recovered (ug/ml) | % Recovery | AVG | STDEV | % RSD |
|---|---|---|---|---|---|---|---|---|
| 80% | Rep 1 | 39.88 | 1377417 | 39.33 | 98.61 | 98.95% | 0.295692 | 0.30 % |
| | Rep 2 | | 1384571 | 39.53 | 99.12 | | | |
| | Rep 3 | | 1384571 | 39.53 | 99.12 | | | |
| 100% | Rep 1 | 49.85 | 1745407 | 49.83 | 99.96 | 99.98% | 0.053181 | 0.05 % |
| | Rep 2 | | 1745102 | 49.82 | 99.95 | | | |
| | Rep 3 | | 1746841 | 49.87 | 100.04 | | | |
| 120% | Rep 1 | 59.82 | 2083617 | 59.49 | 99.44 | 99.51% | 0.073901 | 0.07 % |
| | Rep 2 | | 2084512 | 59.51 | 99.49 | | | |
| | Rep 3 | | 2086632 | 59.57 | 99.59 | | | |

Accuracy for GLIBENCLAMIDE was performed in triplicates and it was observed that the method was accurate for the range 80%, 100% and 120%. The relative standard deviation for 80%, 100% and 120% were 0.56%, 0.16% and 0.11% respectively. The accuracy determined the methods ability to analyses different concentration of drug in solution accurately. The accuracy data is shown in table 13.

Table No.13: Accuracy data for Glibenclamide

| Sample ID | Reps | Spiked Conc (ug/ml) | Area | Amount Recovered (ug/ml) | % Recovery | % AVG | STDEV | %RSD |
|---|---|---|---|---|---|---|---|---|
| 80% | Rep 1 | 39.88 | 1172396 | 39.69 | 99.52 | 99.92% | 0.556822 | 0.56% |
| | Rep 2 | | 1174251 | 39.75 | 99.68 | | | |
| | Rep 3 | | 1184571 | 40.10 | 100.55 | | | |
| 100% | Rep 1 | 49.85 | 1472655 | 49.85 | 100.01 | 100.03 % | 0.157728 | 0.16% |
| | Rep 2 | | 1475455 | 49.95 | 100.20 | | | |
| | Rep 3 | | 1470845 | 49.79 | 99.88 | | | |
| 120% | Rep 1 | 59.82 | 1766178 | 59.79 | 99.95 | 99.98% | 0.10986 | 0.11% |
| | Rep 2 | | 1768974 | 59.88 | 100.11 | | | |
| | Rep 3 | | 1765243 | 59.76 | 99.90 | | | |

### 3.5. Inter and Intraday Precision

Intra and inter day precision study was performed and reported the % RSD change in peak area of the APIs at different time points. The acceptance criteria is to have %RSD of peak area <2%. The Results are given in Table 14.

**Table No. 14. Inter and Intraday Precision**

| Intra - day Precision | | | | |
|---|---|---|---|---|
| **Sample ID** | **Glipizide** | | **Glibenclamide** | |
| | **RT** | **Area** | **RT** | **Area** |
| **Morning** | 4.91 | 1745407 | 9.45 | 1472655 |
| **Evening** | 4.91 | 1723654 | 9.45 | 1471222 |
| **%RSD** | - | 0.89% | - | 0.07% |
| **Inter - day Precision** | | | | |
| **Sample ID** | **Glipizide** | | **Glibenclamide** | |
| | **RT** | **Area** | **RT** | **Area** |
| **Day 1** | 4.91 | 1745407 | 9.45 | 1472655 |
| **Day 2** | 4.91 | 1736547 | 9.45 | 1462531 |
| **%RSD** | - | 0.36% | - | 0.49% |

### 3.6. Robustness

Robustness is done to check how deviating the method is with respect to its critical parameters. All over the world, the equipment is calibrated before use, but to know if the method is robust, changes were done in column temperature and Wavelength as shown in table 15 and 16.

Table No. 15: Robustness data for GLIPIZIDE & GLIBENCLAMIDE with changes in Column Oven Temperature

| Column Oven Temperature | | | | | |
|---|---|---|---|---|---|
| **Condition** | **Sample ID** | **Glipizide** | | **Glibenclamide** | |
| | | **RT** | **Area** | **RT** | **Area** |
| **Increase** | **WS** | 4.91 | 1745236 | 9.45 | 1435874 |
| **Normal** | **WS** | 4.91 | 1745407 | 9.45 | 1472655 |
| **Decrease** | **WS** | 4.91 | 1723257 | 9.45 | 1452455 |
| **%RSD** | - | 0.00 | 0.73% | 0.00 | 1.27% |

Table No. 16: Robustness data for GLIPIZIDE & GLIBENCLAMIDE with changes in Wavelength

| Wavelength Change (nm) | | | | | |
|---|---|---|---|---|---|
| Condition | Sample ID | Glipizide | | Glibenclamide | |
| | | RT | Area | RT | Area |
| Increase | WS | 4.91 | 1702547 | 9.45 | 1436521 |
| Normal | WS | 4.91 | 1702547 | 9.45 | 1472655 |
| Decrease | WS | 4.91 | 1715421 | 9.45 | 1456234 |
| %RSD | - | 0.00 | 0.44% | 0.00 | 1.24% |

There was no significant change in retention time of GLIPIZIDE & GLIBENCLAMIDE peak with change in Column oven Temperature and Wavelength as the RSD is within the acceptance criteria according ICH guidelines.

### 3.7. % Assay:

Based on the validated method, assay was carried out on marketed formulation. The assay results are mentioned in table no. 17

**Table No. 17: Market product Assay**

| Sample ID | Glipizide | | | Glibenclamide | | |
|---|---|---|---|---|---|---|
| | RT | Area | % Assay | RT | Area | % Assay |
| Blank | - | - | - | - | - | - |
| Glipizide WS | 4.91 | 1746102 | - | - | - | - |
| Glibenclamide WS | - | - | - | 9.45 | 1472341 | - |
| MIX WS | 4.91 | 1745407 | - | 9.45 | 1472655 | - |
| Drug Product | 4.91 | 1744584 | 99.95% | 9.45 | 1471457 | 99.92% |

The assay was found to be between 99 to 101% for market formulations.

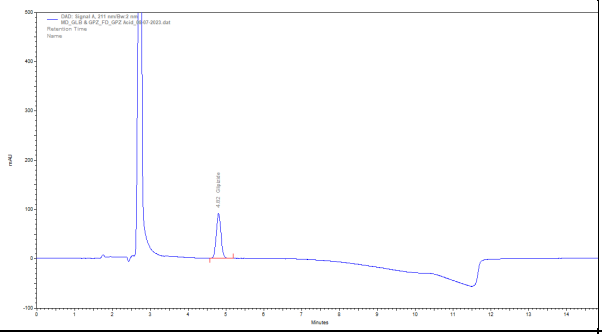
### 3.8. Forced degradation

Based on the Forced degradation results of GLIPIZIDE & GLIBENCLAMIDE as shown in Table 18, in oxidation condition Glipizide was stored at room temperature and at 60°C but no degradation was observed in either of the condition, whereas, Glibenclamide showed degradation of 5.30%. In acidic condition the degradation for Glipizide & Glibenclamide was found to be 24.78% & 7.55% respectively. In basic condition Glipizide showed no degradation & Glibenclamide was found to be degraded up to 37.76%. Glibenclamide showed no degradation in heat and Photolytic condition, whereas, Glipizide degraded up to 4.71% in Heat but no degradation was found in Photolytic condition.

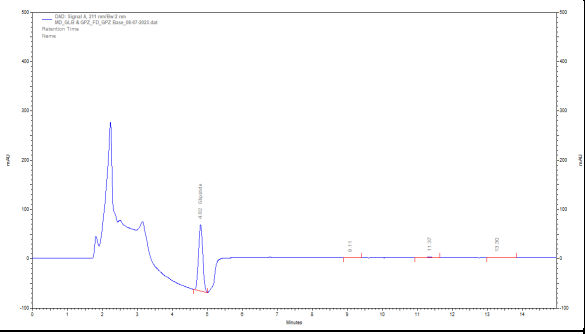
**Table No. 18: Forced degradation in GLIPIZIDE & GLIBENCLAMIDE**

| Sample ID | Glipizide | | | Glibenclamide | | |
|---|---|---|---|---|---|---|
| | Area | % Assay | % Degradation | Area | % Assay | % Degradation |
| Control | 2166313 | 100.00 | - | 4071648 | 100.00 | |
| Peroxide | 2199726 | 101.54 | No Degradation | 3855921 | 94.70 | 5.30 |
| Peroxide + Heat at 60°C | 2177008 | 100.49 | No Degradation | - | - | - |
| Acid | 1629549 | 75.22 | 24.78 | 3764278 | 92.45 | 7.55 |
| Base | 2189020 | 101.05 | No Degradation | 2534254 | 62.24 | 37.76 |
| Heat | 2064174 | 95.29 | 4.71 | 4112986 | 101.02 | No Degradation |
| Light | 2181650 | 100.71 | No Degradation | 4133087 | 101.51 | No Degradation |

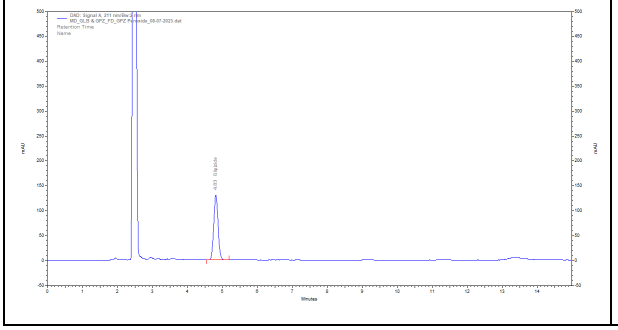
**a. Acid hydrolysis**



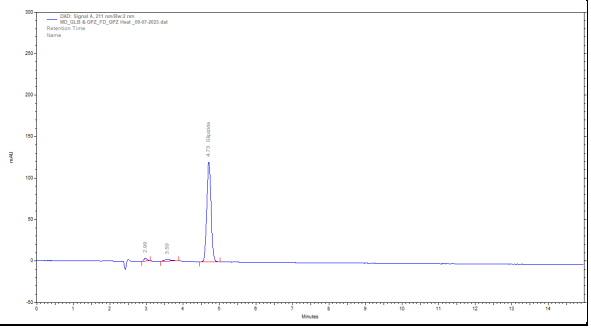
**b. Base Hydrolysis**



**c. Oxidation**



**d. Heat Degradation**



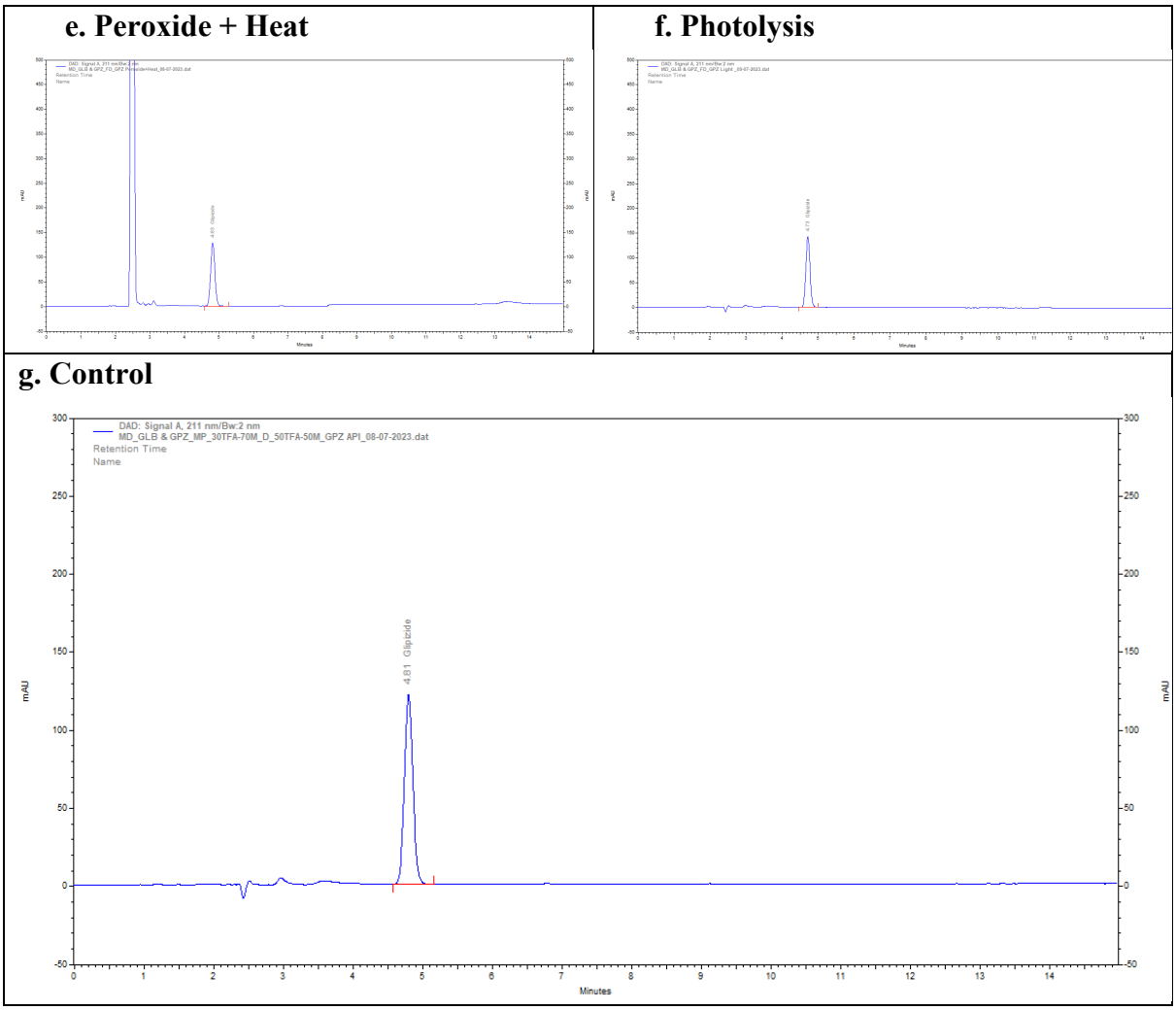
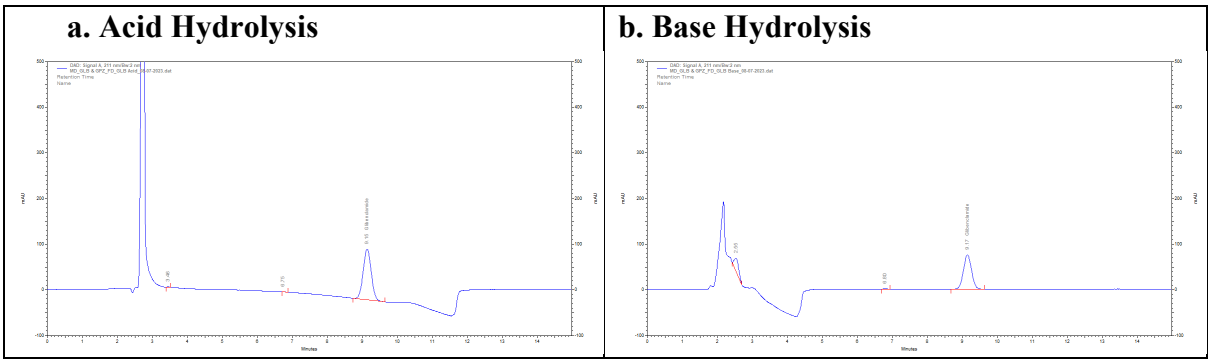



**Figure No. 7. : Forced Degradation in Glipizide a) Acid Hydrolysis, b) Base hydrolysis, c) Oxidation, d) Heat Degradation e) Peroxide degradation with heat, f) Photolysis and g) Control.**



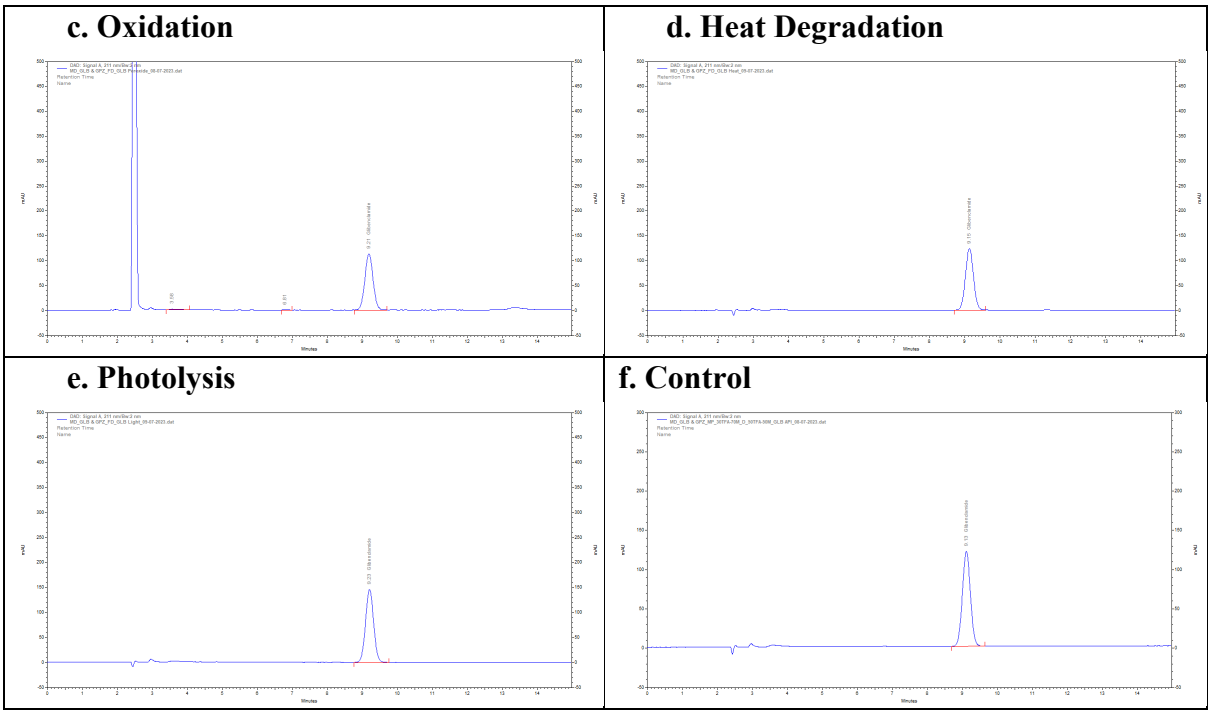



**Figure No. 8. : Forced Degradation in Glibenclamide a) Acid Hydrolysis, b) Base hydrolysis, c) Oxidation, d) Heat Degradation e) Photolysis and f) Control.**

## Conclusion

In this research article, a precise and accurate method was developed based on method developed technique for estimation of GLIPIZIDE & GLIBENCLAMIDE in bulk drugs and formulation by RP-HPLC technique. The developed method was validated for accuracy, precision and robustness. From Forced degradation studies, it was found that Glipizide degraded in acidic and heat conditions and Glibenclamide degraded in oxidation, acid and base hydrolysis. As there was no published method for analysis of these drug in a single method by RP-HPLC, therefore this method can be employed for the simultaneous analysis of Glipizide and Glibenclamide.

## References

1. Costello RA, Nicolas S, Shivkumar A. Sulfonylureas - Stat Pearls - NCBI Bookshelf [Internet]. Stat Pearls; 2023 [cited 2023 Sept 15]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK513225/
2. Nanovskaya TN, Patrikeeva S, Hemauer S, Fokina V, Mattison D, Hankins GD, et al. Effect of albumin on transplacental transfer and distribution of rosiglitazone and Glyburide. The Journal of Maternal-Fatal & Neonatal Medicine. 2008;21(3):197–207. doi:10.1080/14767050801929901.
3. Glipizide [Internet]. U.S. National Library of Medicine; [cited 2023 Sept 15]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Glipizide.
4. Glyburide [Internet]. U.S. National Library of Medicine; [cited 2023 Sept 16]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Glyburide.
5. Rao BU, Nikalje AP. Determination of glipizide, Glibenclamide and Glimepiride in a tablet dosage form in the presence of metformin hydrochloride by ion pair –reversed

phase liquid chromatographic technique. Journal of Analytical & Bioanalytical Techniques. 2010;01(02). doi:10.4172/2155-9872.1000105.

6. Lakshmi KS, Rajesh T. Development and validation of RP-HPLC method for simultaneous determination of glipizide, rosiglitazone, pioglitazone, Glibenclamide and Glimepiride in pharmaceutical dosage forms and human plasma. Journal of the Iranian Chemical Society. 2011;8(1):31–7. doi:10.1007/bf03246199.
7. R. Tengli A. Method development and validation of Metformine, pioglitazone and Glibenclamide in tablet dosage form by using RP-HPLC. Biochemistry & Analytical Biochemistry. 2013;02(02). doi:10.4172/2161-1009.1000130
8. AbuRuz S, Millership J, McElnay J. The development and validation of liquid chromatography method for the simultaneous determination of metformin and glipizide, gliclazide, Glibenclamide or glimepiride in plasma. Journal of Chromatography B. 2005;817(2):277–86. doi:10.1016/j.jchromb.2004.12.018.
9. Venkatesh P, Harisudhan T, Choudhury H, Mullangi R, Srinivas NR. Simultaneous estimation of six anti-diabetic drugs—Glibenclamide, gliclazide, glipizide, pioglitazone, repaglinide and rosiglitazone: Development of a novel HPLC method for use in the analysis of pharmaceutical formulations and its application to human plasma assay. Biomedical Chromatography. 2006;20(10):1043–8. doi:10.1002/bmc.635
10. Mallu UR, Pyreddy VR, Penumajji SS, Bobbarala V. Single and high resolution RP-HPLC method for the determination of six anti diabetic drug products. Journal of Pharmacy Research. 2011;4(4):1209–12.
11. Atif M, Khalid SH, Onn Kit GL, Sulaiman SAS, Asif M, Chandersekaran A. Development and validation of RP-HPLC-UV method for the determination of glipizide in human plasma. Journal of Young Pharmacists. 2013;5(1):26–9. doi:10.1016/j.jyp.2013.01.005.
12. Pani NR, Acharya S, Patra S. Development and validation of RP-HPLC method for quantification of glipizide in biological macromolecules. International Journal of Biological Macromolecules. 2014; 65:65–71. doi:10.1016/j.ijbiomac.2014.01.007
13. Haq N, Alanazi FK, Alsarra IA, Shakeel F. Rapid Analysis of Glibenclamide Using an Environmentally Benign Stability-Indicating RP-HPLC Method. Iran J Pharm Res . 2014;13(3):863–72.
14. Jayanthi M, Thirunavukkarasu SV, Nagarajan V, Elangovan S, Raja S. Development and Validation of RP-HPLC Method for Determination of Glibenclamide in Pharmaceutical Dosage Forms. International Journal of ChemTech Research. 2012;4(2).
15. Kumar A, Srirangam P, Kumar YP, Madhu B. Development and validation of HPLC method for the determination of Glibenclamide in rat serum. International Journal of Pharma and Bio Sciences. 2011;2(1):478–85.