# ESTIMATE GLICLAZIDE SIMULTANEOUSLY IN TABLET DOSAGE FORM BY RP-HPLC

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***ABSTRACT :*** *The objective of the present research work was to develop a innovative, simple, and economic method for estimation of Gliclazide in bulk and dosage form by RP-HPLC.The chromatographic conditions were performed on Phenomenex Luna C18, 100A, 5µm, 250mmx4.6mm i.d.as stationary phase and mobile phase was prepared with a mixture of Acetonitrile : Phosphate Buffer(4.6)= 35:65 flow 1.0 ml/min, with Injection Volume 20µl, at detection wavelength 229 nm and run time at 7.0 min.The analytical method is valid for estimation of Gliclazide over a range of 0 µg/ml–60µg/ml. The results of system suitability test, linearity, precision and accuracy, robustness, specificity, LOD and LOQ and stabilities presented in this report are within the acceptance range.A specific, sensitive, economic method estimation of Gliclazide has been developed based on ICH Guidelines with bulk and dosage forms.*
***Key Words:*** *Gliclazide, HPLC, Method Development, ICH, Validation, Accuracy, Precision.*

## I.INTRODUCTION

Gliclazide is an oral antihyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM). It belongs to the sulfonylurea class of insulin secretagogues, which act by stimulating β cells of the pancreas to release insulin. Sulfonylureas increase both basal insulin secretion and meal-stimulated insulin release. Medications in this class differ in their dose, rate of absorption, duration of action, route of elimination and binding site on their target pancreatic β cell receptor. Sulfonylureas also increase peripheral glucose utilization, decrease hepatic gluconeogenesis and may increase the number and sensitivity of insulin receptors. Sulfonylureas are associated with weight gain, though less so than insulin. Due to their mechanism of action, sulfonylureas may cause hypoglycemia and require consistent food intake to decrease this risk. The risk of hypoglycemia is increased in elderly, debilitated and malnourished individuals. Gliclazide has been shown to decrease fasting plasma glucose, postprandial blood glucose and glycosolated hemoglobin (HbA1c) levels (reflective of the last 8-10 weeks of glucose control). Gliclazide is extensively metabolized by the liver; its metabolites are excreted in both urine (60-70%) and feces (10-20%).

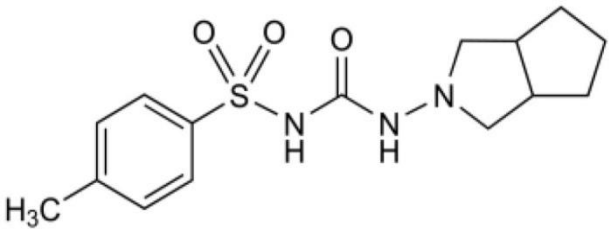



**Fig-1: Structure of Gliclazide**

According to literature survey there is no official method for the estimation of Gliclazide by RP-HPLC in tablet dosage forms. Hence, an attempt has been made to develop new method for the estimation and validation of Gliclazide in tablet formulation in accordance with the ICH guidelines

## II.EXPERIMENTAL

### 2.1 Materials and Methods:

Pharmaceutical grade working standard Gliclazide were obtained from Syncorp Pvt. Laboratories, Hyderabad, India. All chemicals and reagents were HPLC grade and were purchased from S D Fine-Chem Limited & Loba Chemie Pvt Ltd, Mumbai, India

### 2.2 Instrumentation:

The analysis was performed using HPLC (Waters-717 series) with PDA detector and data handling system

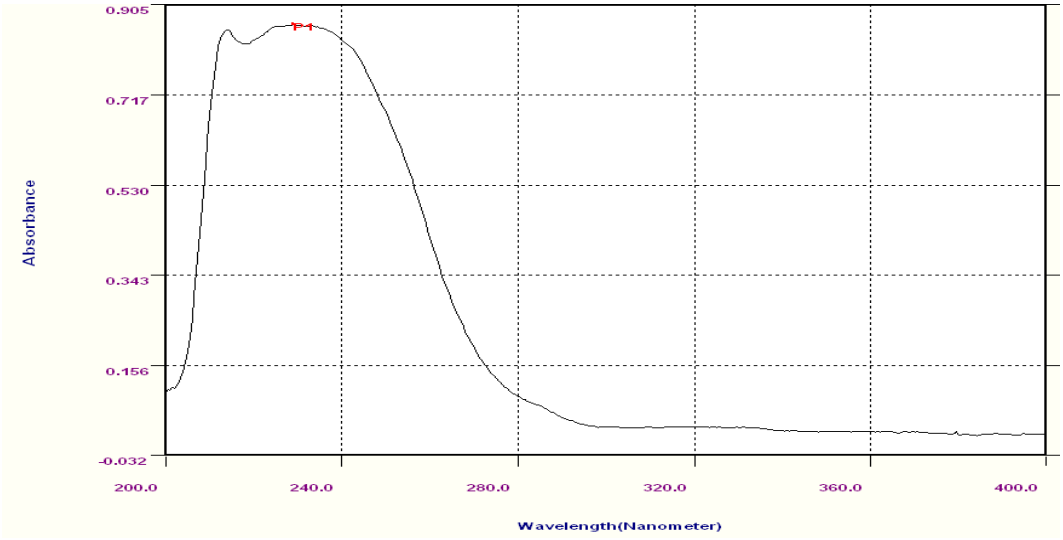
EMPOWER2 software, UV-Visible double beam spectrophotometer (ELICO SL-159), analytical balance 0.1mg Sensitivity (SHIMADZU), pH meter (Labindia), ultra sonicator. The column used is Phenomenex Luna $C_{18}$, 100A, 5µm, 250mmx4.6mm i.d. (as Stationary phase) with the flow rate 1.0ml/min (isocratic).

**2.3 Sample & Standard Preparation for the Analysis**

25 mg of Gliclazide standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.1ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

**2.4 Selection of wavelength**

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. While scanning the Gliclazide solution we observed the maxima at 229 nm.




**Uv spectrum of Gliclazide (229nm)**

**2.5 Method Development**

**2.5.1 Phosphate Buffer Preparation:**

Weigh accurately about 2.72172grams of Potassium dihydrogen orthophosphate was and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water (0.02M). The pH was adjusted to 4.6 with the orthophosphoric acid.

**2.5.2 Mobile Phase Preparation:**

650ml (65%) of above selected buffer and 350 ml of Acetonitrile HPLC (35%) were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.

**2.5.3 Summary of Optimized Chromatographic Conditions:**

The Optimum Chromatographic conditions obtained from experiments can be summarized as below:

**Column** : Symmetry ODS $C_{18}$, 250mm x 4.6mm i.d. and 5µm Particle size
**Mobile Phase** : ACN: Phosphate Buffer (pH=4.6) = 35:65
**Flow rate** : 1.0ml/min
**Detection Wavelength:** 229 nm
**Auto sampler Temperature:** Ambient
**Injection Volume** : 20µl
**Run time** : 6.0minutes
**Column Temperature:** Ambient

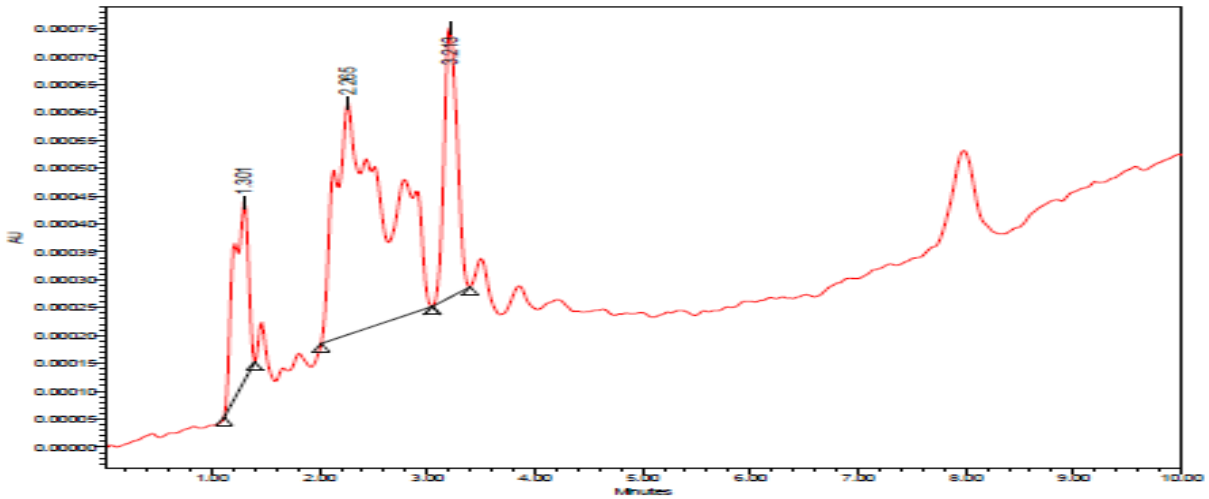



**Fig-: Chromatogram for Blank (Mobile phase Preparation)**

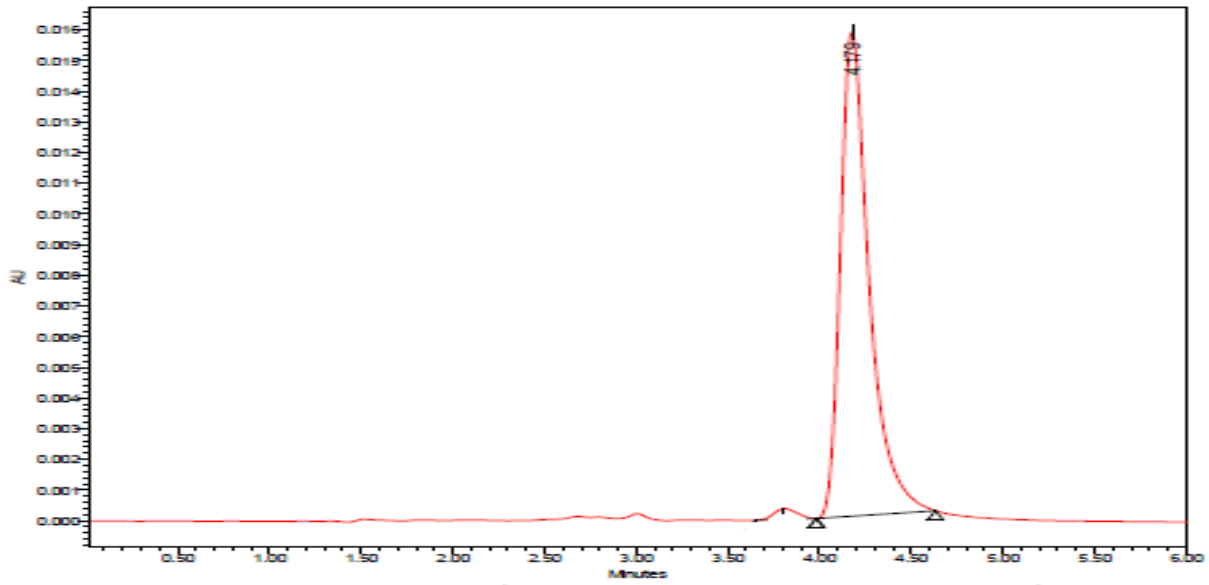



**Fig-: Chromatogram for Optimized Condition (Rt-4.179 minutes)**

**2.6 Method validation:**

**2.6.1 Linearity & Range:**

Calibration standards at five levels were prepared by appropriately mixed and further diluted standard stock solutions in the concentration ranges from 0-60µg/mL for Trandolapril. Samples in triple injections were made for each prepared concentration. Peak areas were plotted against the corresponding concentration to obtain the linearity graphs. Chromatograms of each solution were recorded.

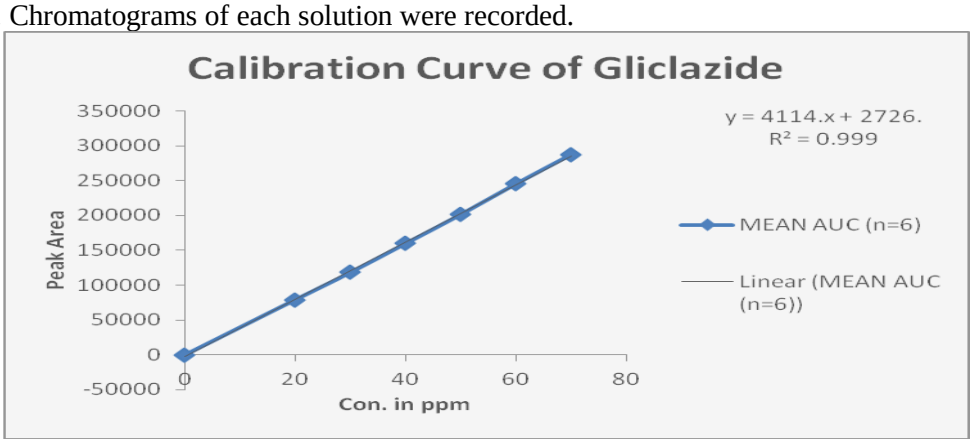


**Table-1: Concentration of Gliclazide**

| CONC.(µg/ml) | MEAN AUC (n=6) |
|---|---|
| 0 | 0 |
| 20 | 79145 |
| 30 | 118346 |
| 40 | 159867 |
| 50 | 201495 |
| 60 | 245468 |
| 70 | 287456 |

**2.6.2. Accuracy:**

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Gliclazide were taken and added to the pre-analyzed formulation of concentration 100µg/ml. From that percentage recovery values were calculated. The results were shown in table-3.

**Table-2: Accuracy Readings**

| Accuracy level | Concentration (µg/ml) | | Peak Area | % Recovery of Pure drug | Statistical Analysis |
|---|---|---|---|---|---|
| | Amount injected | Amount recovered | | | |
| 80 % | 40 | 40.354 | 168745 | 100.885 | Mean= 100.5817%<br>S.D. = 0.284795%<br>R.S.D.= 0.283148% |
| 80 % | 40 | 40.128 | 167816 | 100.32 | |
| 80 % | 40 | 40.216 | 168175 | 100.54 | |
| 100 % | 50 | 50.010 | 208469 | 100.02 | Mean= 100.108%<br>S.D. = 0.081191%<br>R.S.D.= 0.081104% |
| 100 % | 50 | 50.090 | 208797 | 100.18 | |
| 100 % | 50 | 50.062 | 208684 | 100.124 | |
| 120 % | 60 | 60.075 | 249875 | 100.125 | Mean= 99.72533%<br>S.D. = 0.699183%<br>R.S.D.= 0.701108% |
| 120 % | 60 | 60.080 | 249896 | 100.133 | |
| 120 % | 60 | 59.351 | 246897 | 98.918 | |

### 2.6.3. Precision:

#### 2.6.3.1. Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug Gliclazide (API). The percent relative standard deviation was calculated for Gliclazide are presented in the Table-4.

**Table-3: Repeatability Results**

| HPLC Injection Replicates of Gliclazide | Retention Time | Peak Area |
|---|---|---|
| Replicate – 1 | 4.172 | 1235412 |
| Replicate – 2 | 4.175 | 1245876 |
| Replicate – 3 | 4.173 | 1254687 |
| Replicate – 4 | 4.175 | 1254356 |
| Replicate – 5 | 4.170 | 1252415 |
| Replicate – 6 | 4.174 | 1254872 |
| **Average** | **4.173167** | **1249603** |
| **Standard Deviation** | **0.001941** | **7737.994** |
| **% RSD** | **0.046506** | **0.619236** |

#### 2.6.3.2. Intermediate precision:

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Gliclazide revealed that the proposed method is precise.

**Results of intra-assay & inter-assay**

| Conc. Of Gliclazide (API) (µg/ml) | Observed Conc. Of Gliclazide (µg/ml) by the proposed method | | | |
|---|---|---|---|---|
| | Intra-Day | | Inter-Day | |
| | Mean (n=6) | % RSD | Mean (n=6) | % RSD |
| 40 | 40.10 | 0.98 | 39.25 | 0.86 |
| 50 | 49.87 | 0.15 | 50.21 | 0.79 |
| 60 | 60.15 | 0.87 | 59.94 | 0.83 |

### 2.6.4. Method Robustness:

Influence of little changes in optimized chromatographic conditions like changes in flow rate (± 0.1ml/min), mobile phase ratio (±2%), Wavelength of detection (±2nm) and Acetonitrile content in mobile phase (±2%) studied to measure the robustness of the method are also in favour of (Table-36, % RSD < 2%) the developed RP-HPLC method for the analysis of Gliclazide (API).

**Summary of Robustness Test**

| Change in parameter | % RSD |
|---|---|
| Flow (1.1 ml/min) | 0.83 |
| Flow (0.9 ml/min) | 0.26 |
| Temperature ($27^0$C) | 0.46 |
| Temperature ($23^0$C) | 0.58 |
| Wavelength of Detection (227 nm) | 0.079 |
| Wavelength of detection (231 nm) | 0.058 |

**2.6.5. LOD & LOQ:**

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

L.O.D. = 3.3(SD/S).

L.O.Q. = 10(SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

**2.6.6. 6. Method Robustness:**

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2$^0$C), Wavelength of detection (±2nm) & Acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-41, % RSD < 2%) the developed RP-HPLC method for the analysis of Gliclazide (API).

**Table-4: Summary of Robustness Test**

| Change in parameter | % RSD |
|---|---|
| Flow (1.1 ml/min) | 0.83 |
| Flow (0.9 ml/min) | 0.26 |
| Temperature (27$^0$C) | 0.46 |
| Temperature (23$^0$C) | 0.58 |
| Wavelength of Detection (227 nm) | 0.079 |
| Wavelength of detection (231 nm) | 0.058 |

**2.7. ASSAY OF GLICLAZIDE IN DOSAGE FORM:**

**Estimation of GLICLAZIDE in TABLET Dosage Form**

GLICLAZIDE 80 mg

Twenty tablets/Capsules were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 100 mg of drugs were transferred to 100 ml volumetric flask, and 70 ml of Hplc grade methanol was added and solution was sonicated for 15 minutes, there after volume was made up to 100 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with hplc grade methanol. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (1ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system.

The solution prepared was injected in five replicates into the HPLC system and the observations were recorded.

A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded. The data are shown in Table-47.

**ASSAY:s**

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where:

AT = Peak Area of Test obtained with test preparation

AS = Peak Area of Standard obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

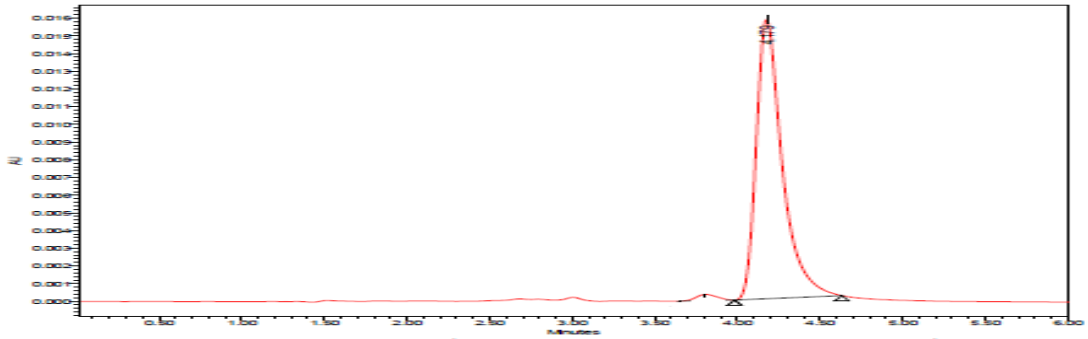
P = Percentage purity of working standard

Assay was performed as described in previous chapter. Results obtained are tabulated below:

**Table-5: Assay of Gliclazide Tablets**

| Brand name of tablets | Labelled amount of Drug (mg) | Mean (±SD) amount (mg) found by the proposed method (n=6) | % PURITY |
|---|---|---|---|
| Azukon Tablet (80 mg)(Torrent Pharmaceuticals Ltd) | 80 | 79.86 (±0.09) | 99.86% |

The assay of Azukon tablets containing Gliclazide was found to be 99.86%.



**Chromatogram for assay standard**

**Table6-: assay chromatogram results of Gliclazide standard**

| S.No. | Drug Name | Rt | Peak Area | Theoretical Plates | Tailing Factor |
|---|---|---|---|---|---|
| 1 | Gliclazide | 4.179 | 1254356 | 3874 | 1.09 |

**Chromatogram for assay sample**

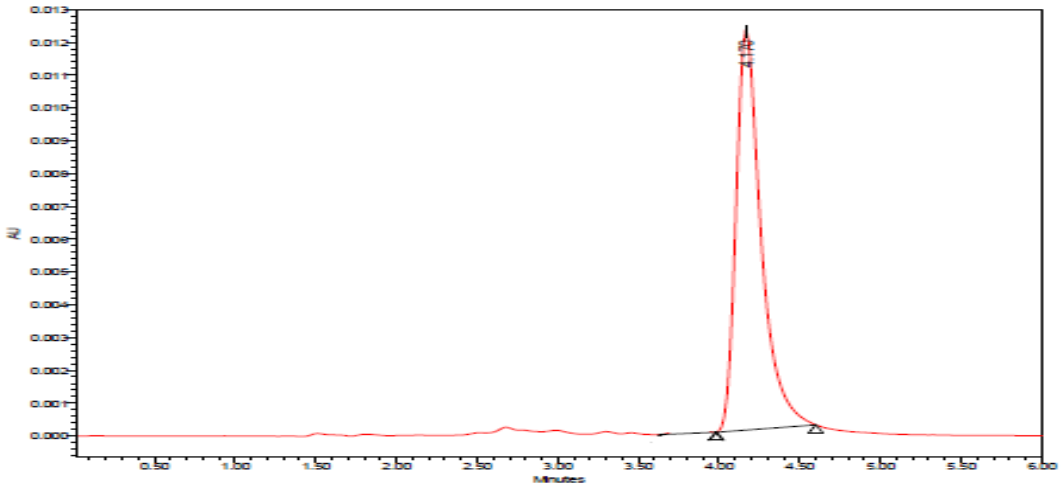


**Table-7: assay chromatogram results of Gliclazide sample**

| S.No. | Drug Name | Rt | Peak Area | Theoretical Plates | Tailing Factor |
|---|---|---|---|---|---|
| 1 | Gliclazide | 4.170 | 1252415 | 3754 | 1.07 |

## III. RESULTS & DISCUSSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Gliclazide, different chromatographic conditions were applied & the results observed are presented in previous chapters.

Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution.

In case of RP-HPLC various columns are available, but here Symmetry ODS C18, 250mm x 4.6mm i.d. and 5µm Particle size column was preferred because using this column peak shape, resolution and absorbance were good.

Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, Acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl).

The drug was found to be Gliclazide is soluble in methanol, DMSO, and acetonitrile, and slightly soluble in ethanol, acetone and practically insoluble in water, which should be purged with an inert gas. Using these solvents with appropriate composition newer methods can be developed and validated.

Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Gliclazide it is evident that most of the HPLC work can be accomplished in the wavelength range of 240-300 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20 µl were found to be the best analysis.

The result shows the developed method is yet another suitable method for assay which can help in the analysis of Gliclazide in different formulations.

## IV. CONCLUSION

A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Gliclazide API.Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The result shows the developed method is yet another suitable method for assay, purity which can help in the analysis of Gliclazide in different formulations.

## REFERENCES


1. Instrumental Methods of Chemical Analysis by B.K. Sharma, pp.75-78, 113-115.
2. Instrumental Methods of Chemical Analysis, Vth Ed., by Galen W. Ewing, 1.
3. Pharmaceutical Analysis, Ist edition, by Takeru Higuchi, Einar Brochmann, Hanffen Hanssen, 1-10.
4. Practical Pharmaceutical Chemistry, IV edition, Volume II, by A.H. Beckett, J.B. Stenlake, 275-298.
5. Quality assurance, worth the effort, Inforum, october2003 volume 7;number.4.
6. Quantitative Analysis of drugs in Pharmaceutical formulation, IIIrd Ed., by P.D. Sethi, pp.1-21, 51-56.
7. Kasture et al, Hand book of Pharmaceutical Analysis, Volume-1.Shetti.P.D, High Performance Liquid Chromatography, 2001, P.11.
8. Validation of Analytical Procedures, Methodology, ICH Harmonized Tripartite Guidelines, 1996.
9. Text on Validation of Analytical Procedures, ICH Harmonized Tripartite Guidelines, 1994.
10. Ravi Shankar, A Text book of Pharmaceutical Analysis, Third edition, page 2.2.
11. Lacy, Charles F, Armstrong, Lora L, Goldman, Morton P, Lance, Leonard L Lexi-Comp's Drug Information Handbook (12th Edition) .Lexi-Comp Inc. ISBN 1-59195-083-X, 2004.