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## Stability Indicating Analytical Method Development and Validation for Assay of Gliclazide in Tablet Dosage Form by using Reverse Phase High Performance Liquid Chromatography

Hamendra Pratap Singh, Keshav Vashistha

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Bhupal Nobles' University, Udaipur (Rajasthan) 313001

**Abstract** A simple, specific, accurate stability indicating RP-HPLC method was developed for assay of gliclazide in pure and tablet form using C18 (150 × 4.6 mm, 5μ) Column and a mobile phase composing of Water, Acetonitrile, methanol TFA and TEA in composition of (40:40:20:0.1:0.1)v/v. The flow rate was 1.5 ml/min and the effluent was monitored at 235 nm. The retention time was 2.3 ±0.1 min. Drug was subjected to acidic, alkali, oxidation, heat and UV and humidity degradation. The degradation studies indicated the susceptibility of drugs to various degradations. The method was statistically validated for accuracy, precision, linearity and forced degradation. Quantitative and recovery studies of the dosage form were also carried out and the % RSD was found to be less than 2. The developed method is simple, rapid and accurate and hence can be used for routine quality control analysis.

**Keywords** Gliclazide, RP-HPLC, Stability indicating

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### Introduction

Pharmaceutical drug products formulated with individual or combination dosage forms. These dosage forms require various qualitative and quantitative analytical methods for the determination of each active ingredient. In this present study developed a single and high resolution RP-HPLC method for the determination of Gliclazide anti diabetic drug products.

Stability is defined as the capacity of a drug substance or drug product to remain within established specifications to maintain its identity, strength, quality, and purity throughout the re-test or expiration dating periods<sup>4</sup>. Stability testing of an active substance or finished product provides evidence on how the quality of a drug substance or drug product varies with time. It is influenced by a variety of environmental factors such as temperature, humidity and light. Knowledge from stability studies enables understanding of the long-term effects of the environment on drugs. Stability testing provides information about degradation mechanisms, potential degradation products, possible degradation pathways of the drug as well as interaction between the drug and excipients in the drug product. The results are applied in developing manufacturing processes and selecting proper packaging, storage conditions, product's shelf life and expiration dates. Because the distribution environment is highly variable, products must be distributed in a manner that ensures product quality will not be adversely affected. The effect of possible temperature and humidity fluctuations, outside of labelled storage conditions, during transportation of drug products can be evaluated on the basis of the stability analysis of the drug.



Gliclazide is an oral anti hyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM). Chemically known as 1- (hexahydrocyclopenta[c] pyrrol-2-(1H)-yl)-3-[(4-methylphenyl) sulfonyl] urea. Sulfonylurea class of drug. Available doses are 30, 60 or 80 mg. This medication is used in conjunction with diet and exercise regimens to control high blood sugar in non-insulin dependent diabetic patients. Controlling high blood sugar helps prevent heart disease, strokes, kidney disease, circulation problems and blindness. Gliclazide undergoes extensive metabolism to several inactive metabolites, mainly methylhydroxygliclazide and carboxygliclazide. The addition of gliclazide to metformin is an effective treatment for patients inadequately controlled on sulfonylurea or metformin alone. A combination of gliclazide with metformin achieves good glycemic control and improves lipid levels with better tolerability profile.

However, the usage of the common formulation of gliclazide can be limited by some kinds of reasons, such as patient's age and renal impairment etc. Recently, gliclazide modified release, as a new formulation administered once daily, has been approved for use in numerous countries worldwide. It has good efficacy, appears of particular benefit in patients previously untreated with oral ant diabetic drugs and is generally well tolerated [2-5]. Sustained release formulation is an important program for new drug research and development to meet clinic needs. The purpose of developing the modified release formulation may be exhibited the following aspects: (1) to increase bioavailability of the drug products; (2) to decrease the times of administration or to prolong duration of administration; (3) to decrease the fluctuation of peak-trough concentration and decrease side effects;(4)to improve the specific distribution of the drug [6-7].

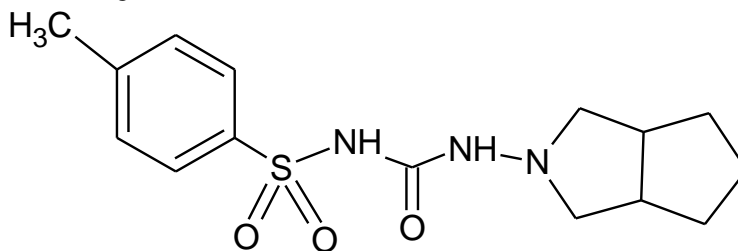


Figure 1: Structure of Gliclazide

Literature surveys revealed that sensitive LC-MS methods are available for analysis of ant diabetic drugs and its metabolites in human plasma and urine. Several HPLC methods have been developed individually and combined dosage forms in human plasma. Even though stability indicating methods are developed for individual Ant diabetic drugs. **Present study aimed for the retention of peak earlier those than reported methods by reverse phase HPLC method.** The method was validated according to the ICH (Q2A 1995) guidelines.

## Experimental Section

### Materials and Methods

#### Reagents and Solutions

High pure (not less than 100%) standards

Gliclazide was used for analysis.

Table 1: List of Material & Solution

Sr. No.	Name of the material	Grade	Make
1	Methanol	HPLC	Merk
2	Acetonitrile	HPLC	Sigma-Aldrich
3	Water	HPLC	Inhouse
4	Triethylamine	Lichrosolv	Merk
5	Trifluoroacetic acid	Uvasol	Merk



**Apparatus**

HPLC system	: Waters (Alliance 2695 module-photo diode array detector)
Balance	: Mettler Toledo analytical balance.
Sonicator	: POWER SONIC420 "Aarkey"
Mobile phase	: Prepared a filtered and degassed mixture of Water, Acetonitrile, Methanol, Trifluoroacetic acid and Triethyl amine (40:40:20:0.1:0.1) v/v
Diluent	: Prepared a filtered and degassed mixture of Water, Acetonitrile (3:2)

**Chromatographic Conditions**

Column	: C18, 150 X 4.6mm, 5 $\mu$
Wave length	: 235nm
Flow rate	: 1.5ml per min
Inj. Volume	: 20 $\mu$ L
Column temp	: 50°C
Run time	: 4 min

**Standard solution preparation**

Weighed and transferred accurately about 10mg of gliclazide working standard into a 50 ml volumetric flask containing 30 ml acetonitrile, after which it was mechanically shaken & sonicated for 15 min, and then diluted to volume with same solvent. Added 5 ml of the resulting solution to 100 ml volumetric flask and made up the volume with diluents.

**Test solution preparation**

Twenty tablets were separately weighed, and their mean weight was determined and powdered. An amount of powdered mass, equivalent to 10 mg of gliclazide was transferred separately into a 50 ml volumetric flask containing 30 ml acetonitrile, after which it was mechanically shaken & sonicated for 15 min, and then diluted to volume with same solvent. Added 5 ml of the resulting solution to 100 ml volumetric flask and made up the volume with diluent

**System Suitability Solution**

The tailing factor of all active peaks in standard solution is not more than 2.0. The percent relative standard deviation for five replicate injections area is not more than 2.0%

**Calculation**

$$\% \text{ of content} = \frac{\text{Area. of test solution} \times \text{Std Concentration} \times \text{average} \times \text{Potency of standard}}{\text{Area of standard solution} \times \text{sample concentration} \times \text{Label claim}}$$

**Results and Discussion****Method Development**

A single and high resolution RP-HPLC method has been developed for the quantification of Gliclazide. In method development stage, trials were performed using various columns, column temperature, and mobile phase composition and run time. Finally, the separation was achieved using Hypersil BDS C18 (150 $\times$ 4.6, 5 $\mu$ ) column with mobile phase composition of Water, Acetonitrile, Methanol, Trifluoroacetic acid and Triethyl amine (40:40:20:0.1:0.1) v/v with simple isocratic program. Diluent and standard solution chromatograms were represented in figure and. The peak is well separated. Peak shape and tailing factor also within the limit.



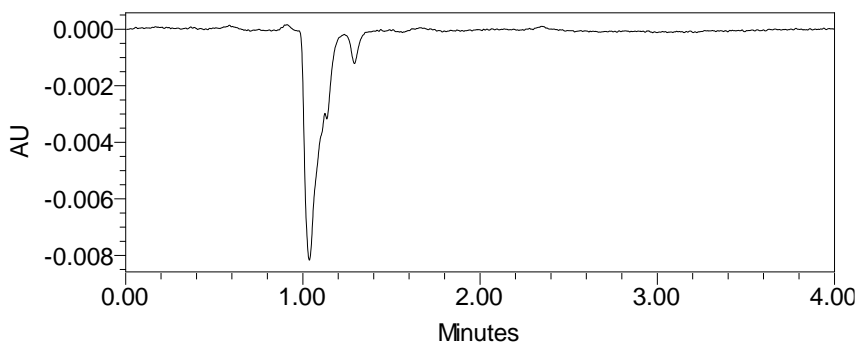


Figure 2: Diluent Chromatogram

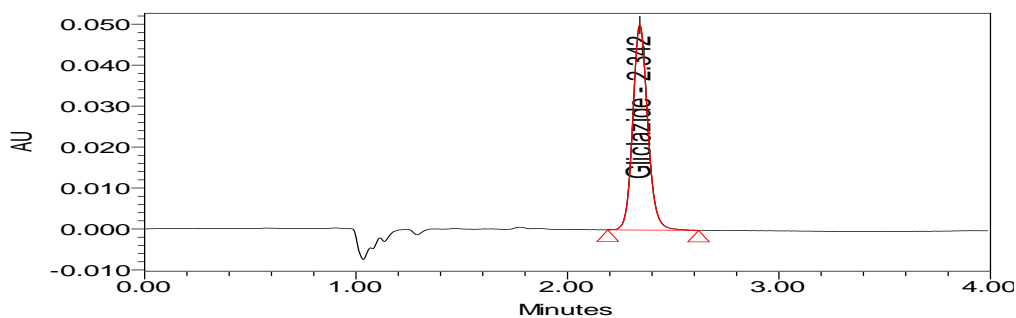
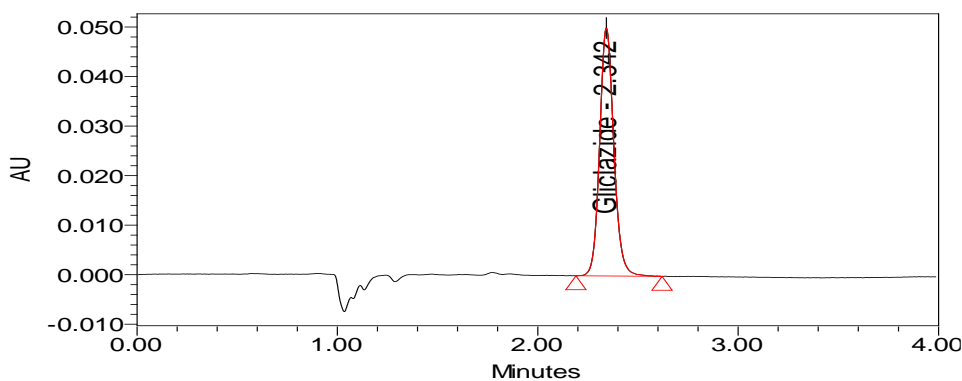


Figure 3: Standard Solution Chromatogram

### System Suitability

System suitability testing is an integral part of method development of analytical procedures.

System suitability test parameters were established. Diluent, standard solution (five replicates each active 10ppm) and test samples were injected in to the chromatographic system and calculated the percent relative standard deviation for area. The results found to be satisfactory and five replicate standard chromatograms represented in figure and tabulated the results in table.



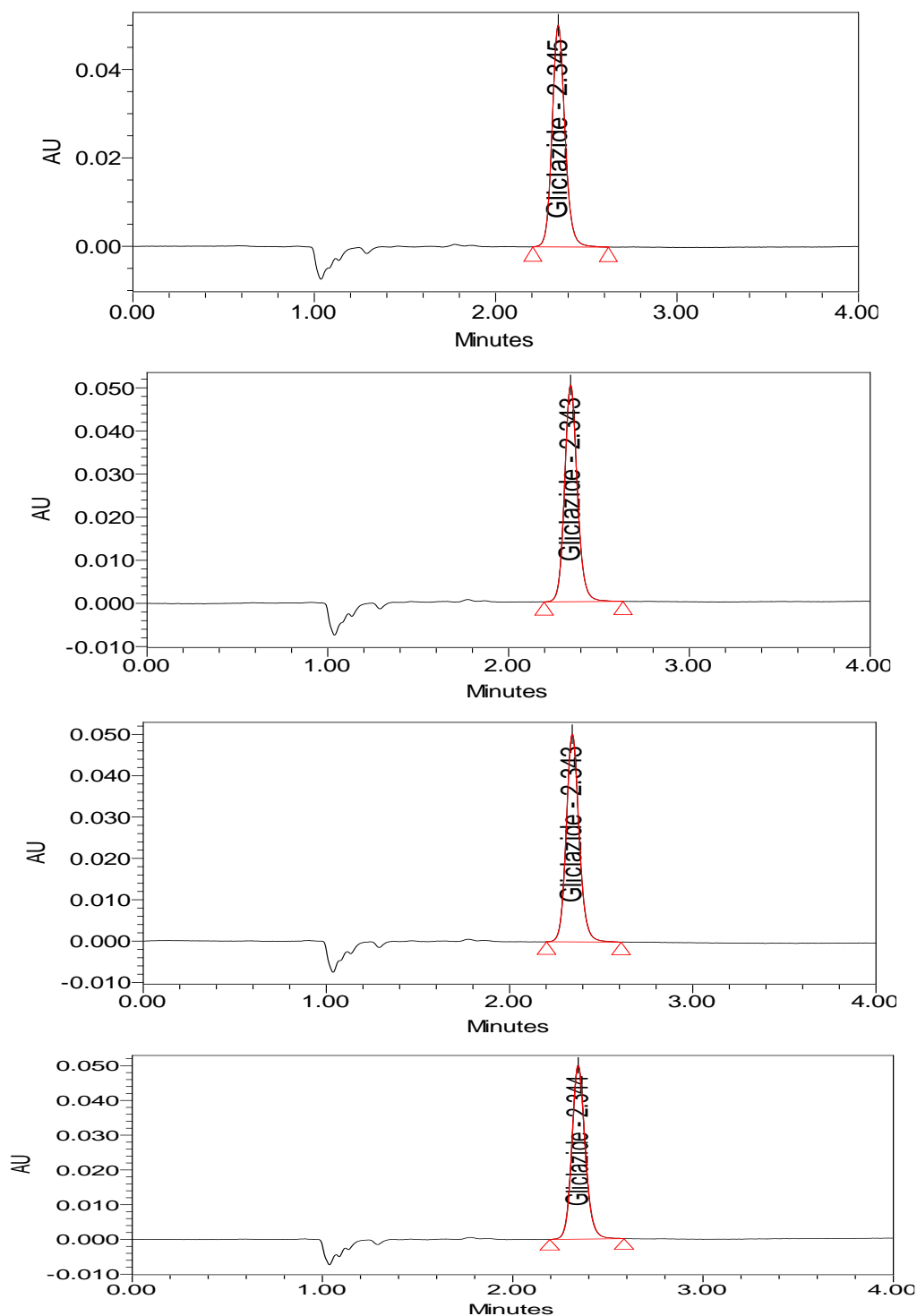


Figure 4: System Suitability Chromatogram

Table 2: System Suitability results (Area % RSD)

Active Ingredient Name	Standard Solution Area					Average	%RSD
	Injection 1	Injection 2	Injection 3	Injection 4	Injection 5		
Gliclazide	246549	246985	247367	246910	246356	246833	0.16

## Method Validation

Validation of an analytical method is a necessary step in controlling the quality of quantitative analysis. Validation can be defined as the process by which it is established, by laboratory studies that the analytical parameters of the method should meet the requirements for the intended analytical applications. Thus, with the background knowledge of linearity, accuracy, precision and robustness of the analytical method, it is relatively easy to derive the confidence and the reliability of the analytical data obtained with it. Validated the developed method as per ICH and FDA (63-67) guidelines with parameters like specificity, precision, accuracy, linearity, solution stability and robustness etc.

## (Specificity) Degradation Studies

Specificity of the method was evaluated with placebo and diluent interference. All active ingredients have no interference with placebo and diluent. Forced degradation of gliclazide tablet sample carried out under different stress conditions (Heat, light, hydrogen peroxide, acid and base) were prepared for further evaluation of the selectivity of the proposed LC method. The selectivity was studied for gliclazide. For preparing acid and base induced degradation, product 5 ml of 0.1 N HCl and 0.1 N NaOH were separately added to 10 mg each of gliclazide equivalent tablet powder and were exposed at 80°C for 3 h. The degraded samples were then neutralized and placed in 50 ml volumetric flask and prepared as described in the sample preparation. For preparing hydrogen peroxide induced degradation, product 5 ml hydrogen peroxide (3% & 5% of 30% v/v) was added to 10 mg each of gliclazide equivalent tablet powder and exposed at room temperature for 3h. The degraded sample was placed in 50 ml volumetric flask and prepared as described in the sample preparation. The forced degradation in acidic, basic and oxidation media was performed in the dark in order to avoid the possible effect of light. For preparing dry heat degradation, product 10 mg each of gliclazide equivalent tablet powder and stored at 80°C for 24 h under dry heat condition in the dark and then cooled to room temperature. The degraded sample solution was prepared as described in the sample preparation. The photochemical stability of the drugs was also studied by exposing the tablet powder to 253nm of UV light by using photo stability chamber. The same procedure was followed as indicated for dry heat degradation. The resulting solutions were used as the degraded sample solution and determined under the described chromatographic condition. Typical chromatograms of all the degraded tablet samples are shown in Figures 2b to d. The degraded samples were compared to a tablet sample without degradation. The spectral homogeneity (with peak purity from 200 to 400 nm) was determined in the forced degraded samples. The threshold was set at  $\geq 0.990$ . The peak purity, peak threshold and percent degradation (Table 1) of gliclazide (10 mg each) demonstrated that the proposed method was able to separate degradants generated during forced degradation studies from gliclazide.

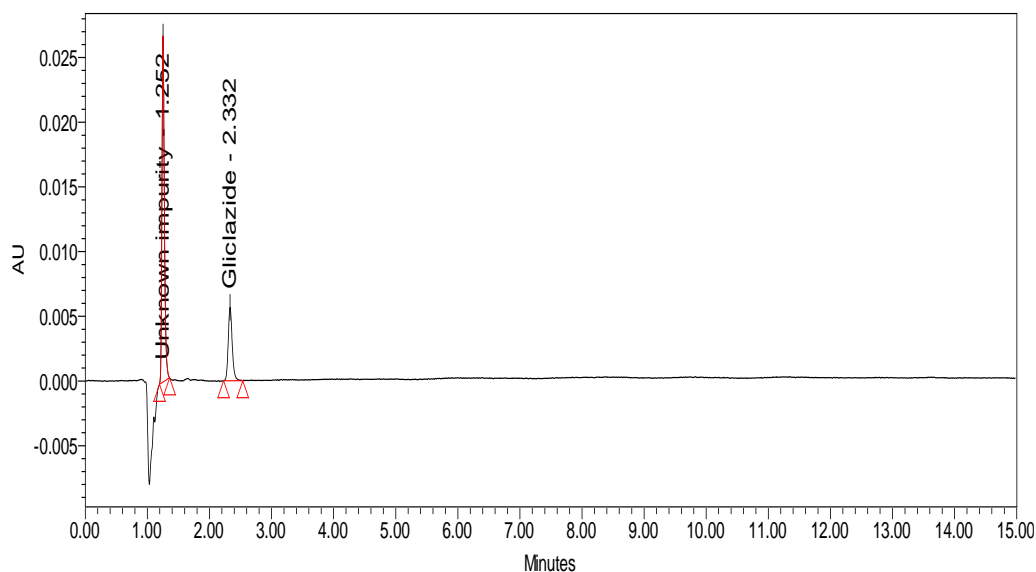


Figure 5: Chromatograms of acid hydrolysis-degraded Gliclazide in tablet sample



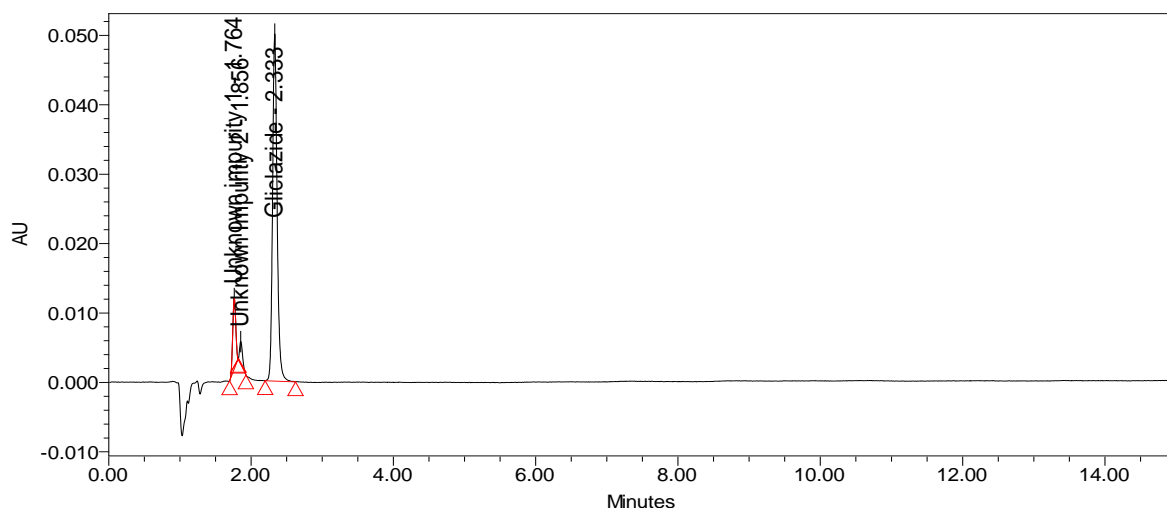


Figure 6: Chromatograms of basic hydrolysis-degraded Gliclazide in tablet sample

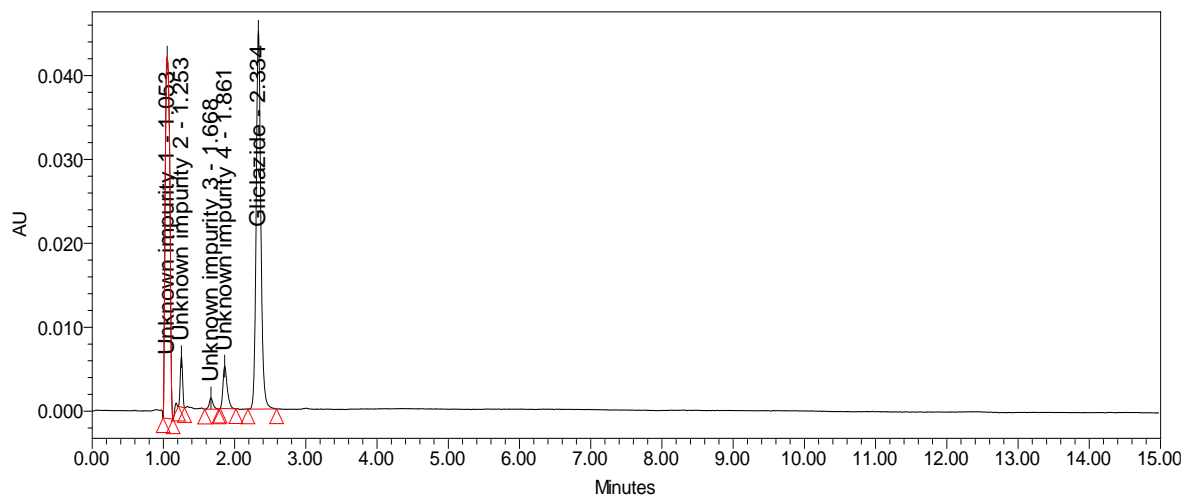


Figure 7: Chromatograms of oxidative-degraded Gliclazide in tablet sample

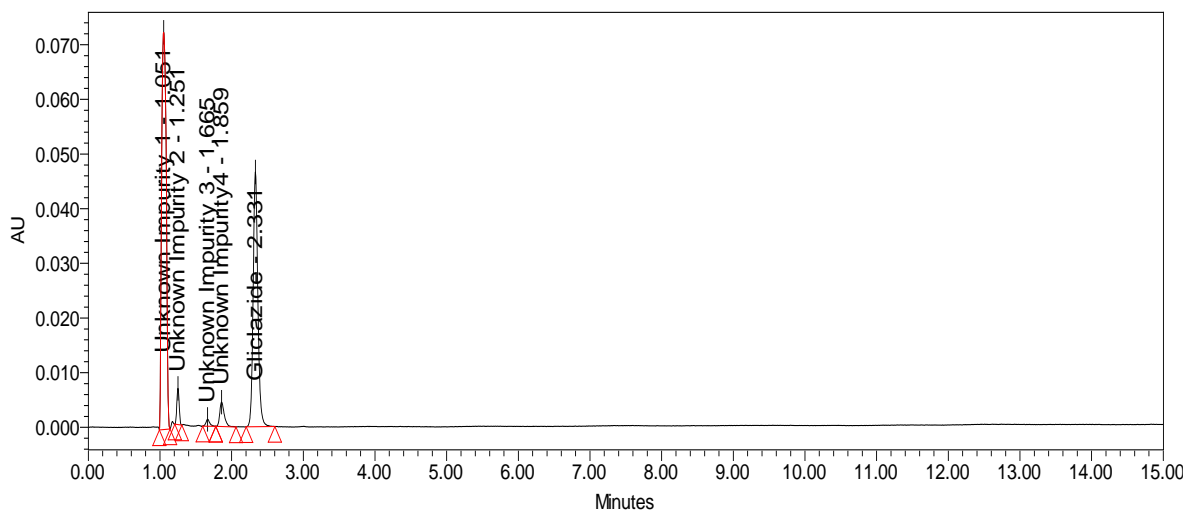


Figure 8: Chromatograms of oxidative-degraded Gliclazide in tablet sample

**Table 3:** Specificity Results

Sample Condition	Purity Angle	Purity Threshold	% Assay	% Degradation
Unstressed	0.257	0.465	101.3	NA
Acidic Hydrolysis	1.986	3.201	9.9	90.2
Basic Hydrolysis	0.277	0.485	81.6	19.4
Oxidative (3% H <sub>2</sub> O <sub>2</sub> ) Hydrolysis	0.470	0.831	78.6	22.6
Oxidative (5% H <sub>2</sub> O <sub>2</sub> ) Hydrolysis	0.435	1.588	81.4	19.7
Heat	0.215	0.432	99.6	1.8
Humidity	0.206	0.410	101.6	-0.3
U.V.	0.217	0.434	99.9	1.3

**Precision**

The repeatability method was evaluated by assaying six samples, prepared as described in the sample preparation. The mean % percentage R.S.D. for assay values of gliclazide were found to be 1.78 %, respectively which is well within the acceptance criteria, that is % R.S.D. should not be more than 2.0%.

**Table 4:** Precision Results

Active Ingredient Name	Sample Preparations						Avg %	% RSD
	Inj 1	Inj 2	Inj 3	Inj 4	Inj 5	Inj 6		
Gliclazide	95.4	99.1	96.0	96.3	94.4	96.0	96.0	1.78

**Linearity**

The linearity of method was evaluated by analyzing five different concentrations of the standard solution ranging from 50 to 150 µg/ml for gliclazide tablets. Calibration curve was constructed by plotting area against concentration and regression equation was computed. The regression equations obtained for gliclazide were  $A = 41911C$  ( $r^2 = 0.9988$ ,  $n = 5$ ). The result shows that an excellent correlation existed between peak area and concentration of gliclazide within the concentration range tested.

**Table 5:** Linearity Results

Active Ingredient Name	Linearity Solution Area					Co-relation Coefficient
	Level 1 5 ppm	Level 2 8 ppm	Level 3 10 ppm	Level 4 12 ppm	Level 5 15 ppm	
Gliclazide	124312	196273	243685	301938	375014	0.999

**Accuracy**

To study the reliability and accuracy of the method, recovery experiments were carried out with a known quantity of the pure drug was added to the placebo sample at the level of 50% to 150% of the test concentration. The contents were determined from the respective chromatograms. The concentration of the drug product in the solution was determined. The mean recoveries were in range of 98.0-102.0 % which shows that there is no interference from excipients. Table represent the recovery results.

**Table 6:** Accuracy (Recovery) Result

Gliclazide			
Level	Recovery (%)	Mean (%)	% RSD
50%	101.6	101.13	0.41
	101.0		
	100.8		
100%	99.3	99.5	0.17
	99.6		
	99.6		
150%	101.7	101.5	0.43
	101.8		
	101.0		





### Robustness

The robustness of the method was determined by analyzing the same samples at standard operating conditions. In all the deliberate varied chromatographic conditions carried out, that is, column temperature, wavelength, and flow rate in mobile phase, the system suitability parameter and % assay for gliclazide from the six replicate injections of the test solution was found to be within the acceptable limits. However, the robustness of the method is established as the percentage deviation from the mean assay value obtained from the precision study, which is less than  $\pm 2\%$ . Table represents the robustness of the method.

**Table 7: Results of robustness study of gliclazide**

Parameter	System Suitability Variation	Initial Precision (%RSD)
Standard condition	-----	0.16
Flow Rate	+10% (1.65ml/min)	1.86
Flow Rate	- 10% (1.35 ml/min)	1.78
Column Temperature	+ 5°C (55°C)	1.53
Column Temperature	- 5 °C (45°C)	1.73
Wavelength	+ 3nm (238nm)	1.48
Wavelength	- 3nm (232nm)	1.48

### Solution stability

Solution stability was studied by evaluating the change in response from initial peak area. The peak of analyte should not differ by more than 2% from initial peak area for the accepted storage time. This study shows that the solution was stable for 24 hours.

**Table 8: Solution Stability (At Room Temperature)**

Time	Standard Solution (% Difference)	Sample Solution (% Difference)
Initial	-	-
2 hrs	0.02	0.06
4 hrs	0.03	0.06
8 hrs	0.07	0.1
16 hrs	0.1	0.1
24 hrs	0.1	0.2

### Conclusion

A reversed phase high performance liquid chromatographic method was developed and validated for the determination of gliclazide in pharmaceutical dosage form as a single component. This chromatographic assay fulfilled all the requirements needed for it to be identified as a reliable and feasible method, including accuracy, recovery and precision data. It is highly accurate, precise and selective. The analytical procedure and its chromatographic run time is less than 4 min. Therefore, the HPLC method can be used as a routine sample analysis for stability study purposes

### References

- [1]. Clinical Guidelines Task Force, International Diabetes Federation, Glucose control: oral therapy, In: Global Guideline for Type 2 Diabetes Brussels, International Diabetes Federation, 2007, Pg. no. 35–38.
- [2]. National Collaborating Centre for Chronic Conditions, Type 2 diabetes: national clinical guideline for management in primary and secondary care, London: Royal College of Physicians, 2008, Pg. no. 86.
- [3]. American Diabetes Association, Standards of medical care in diabetes, Diabetes Care, 2009, 32 (1), S13–61.



- [4]. FDA Guidance for industry, stability testing of drug substances and drug products (draft guidance), Food and drug Administration, Rockville, MD, USA, 1988.
- [5]. Wu Z. Drug stability testing and formulation strategies. *Pharmaceutical development and technology*, 2018, 23(10), 941-941.
- [6]. ICH Good Manufacturing Practice for Active Pharmaceutical Ingredients. International Conference on Harmonization, IFPMA, Geneva, 2000.
- [7]. Carstensen JT, Rhodes CT. A rational approach to stability testing and analytical development for NEC, drug products marketed product stability testing: In: drug stability: principles and Practices, Wolfgang G., Ed, New York, Marcel Dekker, 200, Pg. no. 415-81.
- [8]. Emmanuel OA. *Pharmaceutical manufacturing Hand book*. In: effect of packaging on stability of drugs and drug products, Shyne CD, Shyne CG, Eds, John Wiley & Sons, Inc, New Jersey, 2008, pg. no. 641-686.
- [9]. ICH Harmonized Tripartite Guidance, Q1F: stability data package for registration applications In climatic zones III and IV, EMEA, London, 2003.
- [10]. Pamuru Raja, J. C. Thejaswini, B. M. Gurupadayya and D. Jagadeeswara Reddy, Stability indicating RP-HPLC method for simultaneous determination of anti-diabetic drugs, *Journal of Pharmacy Research*, 2012, vol. 5(3), Pg. no. 1791-1794.
- [11]. Dalia Rashad El-Wasseef, Simultaneous Determination of Metformin, Nateglinide and Gliclazide in Pharmaceutical Preparations Using Micellar Liquid Chromatography, *International Journal of Biomedical Science* 2012, Vol. 8(2), Pg. no. 144-151.
- [12]. Useni Reddy Mallu, Viswanath Reddy Pyreddy, Somasekhar Penumajji and Varaprasad Bobbarala, Single and high resolution RP-HPLC method for the determination of six anti diabetic drug products, *Journal of Pharmacy Research* 2011, Vol. 4(4), Pg No. 1209-1212.
- [13]. Karunanidhi S. Lakshmi and Tirumala Rajesh Separation and Quantification of Eight Antidiabetic Drugs on A High-Performance Liquid Chromatography: Its Application to Human Plasma Assay, *ISRN Pharm.* 2011: 521353.
- [14]. Ketan P. Dadhania, Parthika A. Nadpara, Yadvendra K. Agrawal, Development and validation of spectrophotometric method for simultaneous estimation of Gliclazide and Metformin hydrochloride in Bulk and Tablet dosage form by simultaneous equation method. *International journal of pharmaceutical sciences and research*, 2011; Vol. 2(6): Pg. no. 1559-1563
- [15]. B. Udaykumar Rao, and Anna Pratima Nikalje, Determination of gliclazide in a tablet dosage form in the presence of metformin hydrochloride by ion pair –reversed phase liquid chromatographic technique, *African Journal of Pharmacy and Pharmacology*, 2011, Vol. 5(11), Pg. no. 1331-1337.
- [16]. Ayyappan. J, Umapathi. P, Darlin Quine. S, Validated HPLC method for the fast and sensitive determination of few anti-diabetic drugs residues in support of cleaning validation in formulation area, *International Journal of Pharmacy and Pharmaceutical Sciences*, 2011, Vol 3(5), Pg. no. 371-374.
- [17]. Ms. Shweta Havele, Dr. Sunil Dhaneshwar, Development and Validation of a HPLC Method for the Determination of Metformin Hydrochloride, Gliclazide and Pioglitazone Hydrochloride in Multicomponent Formulation, *Webmed Central, Pharmaceutical Sciences*, 2010, 1(10): WMC001078.
- [18]. Anna Gumieniczek, Anna Berecka Quantitative analysis of gliclazide and glipizide in tablets by a new validated and stability-indicating RPTLC method, *Journal of Planar Chromatography* -2010, Vol. 23, Pg. no. 129-133.
- [19]. YK Agrawal, PJ Gogoi, K Manna, HG Bhatt, VK Jain, A supercritical fluid chromatography/tandem mass spectrometry method for the simultaneous quantification of metformin and gliclazide in human plasma, *Indian Journal of Pharmaceutical Sciences*, 2010, Vol. 72(1), Pg No. 50-57.
- [20]. F.S. Bandarkar & I.S. Khattab, Simultaneous Estimation of Glibenclamide, Gliclazide, and Metformin Hydrochloride in Bulk and Commercial Products using a validated ultra-fast liquid chromatography



- technique, *Journal of Liquid Chromatography & Related Technologies*, Vol. 33(20), 2010, Pg. No. 1814-1830.
- [21]. Kanij Fatema, Md Zakiur Rahman, Tasnuva Haque, Mohammad Abul Kalam Azad, Md Selim Reza, Development and Validation of a Simple Method for Simultaneous Estimation of Metformin Hydrochloride and Gliclazide in Tablets by using Reversed Phase High Performance Liquid Chromatography, *Dhaka University Journal of Pharmaceutical Sciences*, 2010, Vol 9(2), Pg. No. 83-89.
- [22]. Maria-Cristina Ranetti, Mihaela Ionescu, Lavinia Hinescu, Elena Ionica, Valentina Anuta, Aurelina E.Ranetti, Camelia Elena Stecoza, Constanntin Mircioiu, Validation Of a HPLC method for the simultaneous analysis of metformin and gliclazide in human plasma, *FARMACIA*, 2009, Vol. 57(6), Pg No. 728-735.
- [23]. G. Rathinavel, U. umanath et al., RP-HPLC Method for the Simultaneous Estimation of Rosiglitazone and Gliclazide in Tablets, *E-Journal of Chemistry*, 2009, vol. 6(4), Pg. no. 1188-1192.
- [24]. Wang, Irina, Multi-component plasma quantitation of anti-hyperglycemic pharmaceutical compounds using liquid chromatography–tandem mass spectrometry, *Journal of Chromatography B*, 2007, Vol. 856 (1–2), Page. no. 318–327.
- [25]. Jing Yao, Ya-Qin Shi, Zhuo-Rong Li, Shao-Hong Jin, Development of a RP-HPLC method for screening potentially counterfeit anti-diabetic drugs, *Journal of Chromatography B*, 2007, Vol 853,(1–2), Pg. no. 254–259.
- [26]. Gulshan Bansal, Manjeet Singh and Kaur Chand Jindal, Forced Degradation Study on Gliclazide and Application of Validated Stability-Indicating HPLC-UV Method in Stability Testing of Gliclazide Tablets, *Chromatographia*, 2007, Vol. 66, (9-10), Pg. No.751-755,
- [27]. Berecka A, Gumieniczek A, Hopkała H, Development and validation of a new high-performance liquid chromatography method for the determination of gliclazide and repaginate in pharmaceutical formulations, *JAOAC Int.* 2006, vol. 89(2):Pg.No.319-25.
- [28]. P. Venkatesh, T. Harisudhan, Hira Choudhury, Ramesh Mullangi, Nuggehally R. Srinivas, 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, Vol. 20,(10), Page. No. 1043–1048,
- [29]. Guixia Ling, Jin Sun, Jingling Tang, Xiaodi Xu, Yinghua Sun & Prof. Zhonggui He, Liquid Chromatography-Electrospray Ionization Mass Spectrometric Method for Determination of Gliclazide in Human Plasma, *Analytical Letters*, 2006 Vol. 39,(7), Pg. No.1381-1391
- [30]. Jia-Feng Yang, Guang-Li Wei, Rong Lu, Chang-Xiao Liu, Bao-Zhong Zheng, Ping Feng Bioavailability of Gliclazide Sustained Release Tablet in Healthy Volunteers, *Asisn Journal of Pharmacodynamics and Pharmacokinetics*, 2006, Vol.6(2), Pg. No. 153-15
- [31]. S. AbuRuz, J. Millership, J. McElnay The development and validation of liquid chromatography method for the simultaneous determination of metformin and glipizide, gliclazide, glibenclamide or glimperide in plasma, *Journal of Chromatography B*, 2005, Volume 817(2), Pg. no. 277–286
- [32]. Roohi Obaid, Tasneem Ahmed et. Al, Method development for analysis of gliclazide in human plasma by using high performance liquid chromatography, *Pakistan Journal of Pharmaceutical Sciences*, Vol. 15 (2), 2002, Pg. No. 51-56.

