RESEARCH ARTICLE

Development and Validation of a Stability-Indicating RP-HPLC Method for Estimation of Glibenclamidei Bulk and Pharmaceutical Formulation

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ABSTRACT

Glibenclamide is the choice of medicine for diabetes type II. There are numerous methods for estimation; however the methods are either not suitable or expensive. A simple, cheap, precise, and accurate stability-indicating HPLC method was developed and further validated.

The wavelength of 229 nm with a constant flow rate of (Methanol: Mixed Phosphate Buffer, 70:30% v/v (pH 7.3)) 1 mL/min was used during the experimentation. The run time was (run time). The internal standard used was (Internal standard). The sample eluted and showed a sharp peak at 2.1 minutes. It was discovered that calibration curve experiments had a 5–50 ppm linearity. The precision of the developed method was lower than 2% RSD. Recovery studies showed that the method's accuracy and precision ranged from 99.25 to 101.81%. Stress testing was used to determine specificity. The developed method was capable of separating the drug peak from any probable degradation products. There was no evidence of recipient or impurity interference. The method can be used to anticipate how long glibenclamide will be stable. The method was feasible and appropriate for analyzing the presence of glibenclamide in a drug substance as well as a drug product.

Keywords: Glibenclamide, Stress degradation, HPLC, FTIR, Stability, Specificity

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INTRODUCTION

Glibenclamide, also referred to as glyburide, has the chemical name 5-chloro-N-[2-[4-(cyclohexylcarbamoylsulfamoyl) phenyl]ethyl]-2-methoxybenzamide (Figure 1). This compound finds primary application in the management of type 2 diabetes.[1] It is utilized in combination with dietary adjustments and physical activity to assist individuals with type 2 diabetes in regulating their blood glucose levels. Classified as a member of the sulfonylurea medication category, glibenclamide (GBC) functions by augmenting the secretion of insulin from the pancreas, thereby reducing blood glucose levels. It achieves this by obstructing ATP-sensitive potassium channels in pancreatic beta cells, triggering cell membrane depolarization, and facilitating the release of insulin (accompanied by increased intracellular calcium levels in the beta cell).

The underlying goal of this project revolves around the development of a stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) assay. This assay is designed to accurately quantify the presence of this drug in both tablet formulations and bulk quantities.

As per the literature survey, GBC is officially in the Indian Pharmacopoeia (2011) and is assayed by the HPLC method.[2] Few spectrophotometric techniques are reported for determining drugs or drugs in combination with another drug. Very few methods are also noted for determining a stability-indicating RP-HPLC method.

MATERIALS AND METHODS

Instrumentation

An HPLC (JASCO) system with a Hypersil C18 4.6 (i.d.) x 250 mm x 5 m column and a UV sample size of 20 L was used for injection. The combined utilization of the Shimadzu ATX 224 digital weighing balance, BT Ultra Sonicator 48, Systronic pH Meter (802), and Millipore vacuum filter pump (XI 5522050) underscored the comprehensive and meticulous approach employed during method development. These sophisticated instruments ensured precision, consistency, and reliable results, ultimately contributing to the successful establishment of the developed method.

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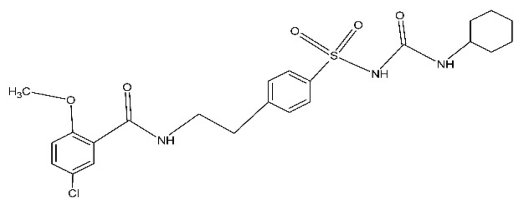


Figure 1: Chemical structure of glibenclamide

Materials and Reagents

The utilization of the pharmaceutical grade working standards of GBC, the GBC tablet formulation (Daolin), AR and HPLC grade chemicals, and Milli-Q water from Mumbai, India, contributed to the precision and accuracy of the analytical methods employed in the study. These materials and reagents ensured the reliability of the results obtained during the analysis of the GBC tablet formulation.

Methods

Fourier–transform infrared spectroscopy

Molecular interactions among the components of the formulation were investigated using an FTIR spectrophotometer. The specific FTIR spectrophotometer employed was the FTIR-8300 model from Shimadzu Corporation, located in Kyoto, Japan. This instrument was utilized to conduct scans on the formulated GBC sample.

For each analysis, individual samples containing 2 mg of GBC were uniformly mixed with potassium bromide (KBR) in a 1:9 mg ratio. These mixtures were then compacted to produce round, transparent discs under a 10 Ton/nm² pressure. Subsequently, the compressed samples underwent a drying process for a duration of 2 hours within a hot air oven maintained at a temperature of 50°C. This step aimed to eliminate any residual moisture from the samples.

Each examination of the dried samples encompassed 45 scans, employing a resolution of 4 cm^{-1} and covering a wavelength range spanning from 4000 to 400 cm^{-1}.[3]

Chromatographic conditions

The multiple trials with combinations of solvent like Acetonitrile: Methanol: Water (6:3:1), Acetonitrile: Water (6:4), Acetonitrile: 25 mM phosphate buffer (pH 3.5) (6:4), exhibited the splitting of peak however peak tailing was observed in phosphate buffer (pH 5) (6.5: 3.5), disodium hydrogen phosphate buffer: methanol (7.5: 2.5). Methanol exhibits the excellent peak of a drug: Mixed phosphate buffer (pH 7.3).

The detection wavelength for the medication was set to be 229 nm. The sample solution was created at the mobile phase. Different solvents of varying polarity were utilized in varying amounts as the mobile phase for chromatogram formation. Finally, the mobile phase with Methanol: Mixed phosphate buffer (70:30% v/v) and pH 7.3 produced the best resolution and crisp peak at a flow rate of 1.0 mL/min(6).

Selection of common solvent

The drug was water-insoluble. However, ethanol, DMSO, chloroform, methanol, and DMF are all soluble. Following the evaluation of drug solubility in several solvents, methanol was chosen as the common solvent for the development of spectrum properties.[4]

Preparation of standard stock solution

Accurately weigh 10 mg of the GBC standard compound using a precise analytical balance. Transfer the weighed 10 mg of GBC into a clean and dry 10 mL volumetric flask. Add 50 mL of methanol to the volumetric flask containing the GBC standard. Place the volumetric flask in an ultrasonic bath (sonicator) and sonicate the solution. Ultrasonication aids in the dissolution and homogenization of the GBC standard in methanol. After sonication, allow the solution to cool to room temperature. Once cooled, carefully adjust the solution volume up to the mark on the 10 mL volumetric flask using additional methanol. This ensures that the final volume is precisely 10 mL. Transfer 1-mL of the prepared solution to a separate 10 mL volumetric flask. Dilute the 1-mL solution to the mark with methanol in the 10 mL volumetric flask. This results in a solution with a concentration of 1000 ppm (parts per million) of GBC.[5,6]

Preparation of working standard solution

The standard working solution of GBC was prepared by dilution from the above standard stock solution at concentrations ranging from 10 ppm. The resultant solution was utilized for UV scanning at 200 to 400 nm.

Validation parameters

To confirm the suitability of the method for its intended purpose, the method was validated following ICH guidelines for system suitability, accuracy, precision, ruggedness, linearity, and range.[7]

- *System suitability test*

System suitability is a pharmacopeial criterion that determines whether the resolution and reproducibility of the chromatographic system are adequate for the analysis. Data was collected from five duplicate injections of a standard laboratory medication combination for the experiments.[7]
The system suitability parameter was created as part of the technique development process and is used to ensure that the chromatographic system performs and repeats properly. The retention duration (R1), capacity factor (K), asymmetry factor, and number of theoretical plates (N) were all measured for five duplicate injections of GBC at a concentration of 10 g/mL.[7]

- ***Resolution***

One of the most important features is that the resolution process is used to determine whether the critical separation is possible under the given parameters.[7]

- ***Retention time***

Retention time is a simplistic factor to determine. The resolution needs to be consistent. This is because values beyond the retention-time window may go unreported by the system under examination.

- ***Pressure***

The suitability testing must be carried out within the prescribed pressure limits. This is done to reduce the wear and tear on

system components. The column is effective; it is also known as band broadening.

- ***Repeatability***

Measurements taken under the same conditions should get the same results.

- ***Plate number***

Efficiency in a certain column is defined as the degree of peak dispersion measured and must have column characteristics. The number of theoretical plates measures efficiency.

- ***Tailing factor***

This is often referred to as the "Symmetry Factor." The tailing factor becomes critical if the peak tailing has the potential to change the method's performance. The type of analysis influences the tailing factor just as it does the plate number.[7]

- *Accuracy*

The accuracy of an analytical method is evaluated based on how closely the test results align with the true value. The assessment of accuracy for the proposed method involved conducting recovery studies.[8]

- *Recovery*

The proposed approach was used for the recovery research. These research studies, known as "multiple level recovery studies," were conducted at three levels.[9] In 10 mL of various volumetric flasks, a precisely weighed quantity of pre-analyzed glibenclamide (10 mg) was taken, and 80, 100, and 120% of glibenclamide pure drug and mobile phase was added. Finally, the volume was marked with the mobile phase and filtered using Whatmann filter paper no. 42, and the necessary dilutions were prepared and sonicated for 30 minutes.[10] The percentage of recovery was then computed as follows:

Formula

$$\%Recovery = \frac{(A - B)}{C} \times 100$$

Here are the rephrased definitions for your variables:

- Represents the percentage of the total drug amount estimated.
- Signifies the percentage of the drug amount found during pre-analysis.
- Denotes the percentage of pure drug that was added.

- *Precision*

The precision of an analytical procedure refers to the level of concordance observed among individual test outcomes. This was ascertained by performing duplicate estimations on a laboratory mixture and quantified using the standard deviation and relative standard deviation of a sequence of observations.[4]

- *Ruggedness*

The ruggedness studies have been carried out in only two separate conditions: different days and different analysts.

The intraday and interday (different days) studies were carried out by producing a 10 µg/mL solution of GBC and analyzing the peak area after 3 hours. Interday and intraday intervals. Analyst investigations were conducted intraday, interday, and on other occasions. The intraday study used 10 g/mL GBC concentrations on the same day, whereas the interday study used different days[11] and distinct analyst study was performed utilizing sample solution generated by two different analysts, and the identical technique as mentioned earlier was followed.

Stress Degradation Studies

Acid hydrolysis

Weigh 50 mg of glibenclamide in bulk accurately. Dissolve the weighed glibenclamide in 50 mL of mobile phase in a volumetric flask. In 5 mL of the produced glibenclamide solution in a 250 mL dry round-bottom flask. Dilute this solution with 50 mL of 0.1 N HCl. This creates an acidic environment for hydrolysis. Set up a reflux system for the round-bottom flask. Heat the reaction mixture to 60°C for 3 hours. Take samples from the reaction mixture during reflux at 0, 1, 2, and 3 hour intervals. Dilute each extracted sample with the mobile phase to a 10 g/mL concentration. Mix the diluted samples thoroughly. To eliminate any particle debris or insoluble compounds, filter each mixed sample through Whatman filter paper no. 42. Approximately 20 µL of each filtered sample should be injected into the HPLC system. Using the HPLC system, separate and quantify the components contained in the samples. Record the chromatogram's peak regions for each time interval. This investigation gives useful information regarding glibenclamide's stability under acidic conditions and aids in determining its susceptibility to degradation.[12]

Alkaline hydrolysis

Precisely measured 50 mg of glibenclamide bulk drug was dissolved in a volumetric flask containing 50 mL of the mobile phase. Subsequently, 5 mL of this prepared solution underwent dilution by combining it with 50 mL of 0.1 N NaOH within a dry 250 mL round bottom flask (RBF). The resulting reaction mixture was subjected to reflux for a duration of 3 hours at a temperature of 60°C. Samples were extracted from the mixture at intervals of 0, 1, 2, and 3 hours, and these samples were further diluted using the mobile phase to attain a concentration of 10 µg/mL. After thorough mixing, the samples were passed through Whatman filter paper no. 42 (size 13) to ensure clarity and purity.[13]

Oxidation studies

Precisely measure out 50 mg of glibenclamide bulk drug and dissolve it in a 50 mL volumetric flask using the mobile phase. Ensure the flask is filled to the mark to achieve the desired volume. Extract 5 mL of this solution and place it into a volumetric flask. Dilute this solution by combining it with a 3% hydrogen peroxide solution within a 50 mL volumetric flask. The resulting mixture, containing the bulk drug glibenclamide, is left to stand for a period of four hours for investigation. Following the four-hour incubation period, extract samples at intervals of 0, 1, 2, and 3 hours. Dilute each extracted sample

with the mobile phase to reach a 10 µg/mL concentration. After thorough mixing, filter the samples through Whatman filter paper no. 42 to ensure clarity and purity. For analysis, inject approximately 20 µL of each filtered sample into the high-performance liquid chromatography (HPLC) system. This action provides chromatograms that display peak areas corresponding to different sample components. The remaining percentage of the drug is calculated using a specific formula designed for the bulk drug, which considers factors such as the initial drug concentration and the peak areas obtained from the chromatograms.[14,15]

Photolytic degradation (UV Light)

As a sample, a sufficient amount of glibenclamide bulk drug was spread on the petri plates. The petri-plate was placed inside the photostability chamber under UV light exposure as per ICH guideline for 12 hours. The samples were withdrawn at 0, 8 and 12 hour for analysis and further diluted to get the 10 µg/mL concentration with mobile phase. Mixed well and filtered through Whatmann filter paper no. 41. The peak area of chromatogram was read out by injecting about 20 µL of each sample into the HPLC system.[11]

Thermal stability studies (Dry heat)

50 mg bulk drug glebenclamide was placed in an oven for 12 hours at 50°C then 10 µg/mL solution of was prepared and peak area of the chromatogram was read out by injecting about 20 µL of sample into the HPLC system.

Limit of detection (LoD) and limit of quantitation (LoQ)

Utilizing the gradient and standard deviation of the y-intercept, the limit of detection (LoD) and limit of quantitation (LoQ) for GBC were determined through linear regression analysis employing the following calculations:

For LoD: LoD = 3.3 * (Q / S)

For LoQ: LoQ = 10 * (Q / S)

In these formulas, S represents the slope of the calibration curve, while Q stands for the standard deviation of the y-intercept.[16]

RESULTS AND DISCUSSION

The melting point was used to verify the GBC's quality and purity, and the results showed a MP range of 168 to 170°C were in accordance with COA. GBC had an absorption maximum in the mobile phase at 229 nm, as shown in Figure 2. Infra-red spectral analysis confirmed the drug purity by its distinguishing peaks shown in Figure 2.

The FTIR spectrum illustrates distinct stretching vibrations: 3345.47 cm^{-1} signifies the N-H stretching of a secondary amine, 3129.66 cm^{-1} corresponds to C-H aromatic stretching, 2999.57 and 2956.4 cm^{-1} represents C-H aliphatic stretching, 1714.60 cm^{-1} signifies C=O stretching, 1613.17 cm^{-1} corresponds to C=C aromatic stretching, and 824.56 cm^{-1} indicates C-Cl stretching (Figure 3).

An HPLC system from JASCO was employed for the HPLC analysis, utilizing a Hypersil C18 column with dimensions of 4.6 mm (i.d.) x 250 mm x 5 µm. A sample volume of 20 µL

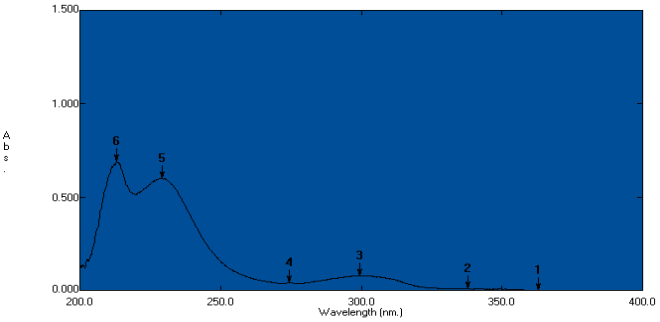


Figure 2: The absorption spectrum of glibenclamide in the mobile phase

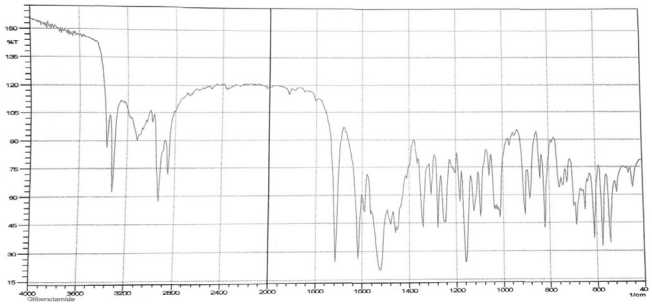


Figure 3: The FTIR spectrum of glibenclamide

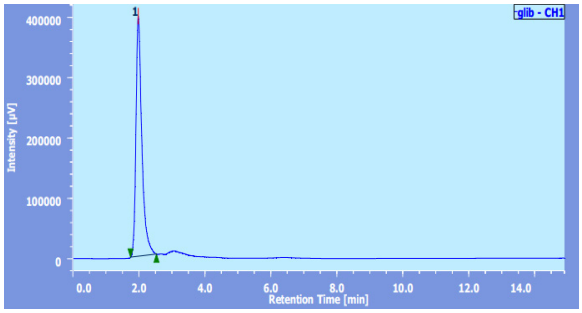


Figure 4: Chromatogram of glibenclamide

was used, and the detection wavelength was set at 229 nm to detect the drug. The mobile phase was prepared using the sample solution, initially experimenting with solvents of varying polarities in different proportions for chromatogram development. Eventually, the mobile phase composed of Methanol: Mixed phosphate buffer (70:30% v/v) with a pH of 7.3 and a flow rate of 1.0 mL/min yielded the highest resolution and well-defined peaks. Figure 4 showcases a chromatogram of glibenclamide with a retention time of 2.1 minutes.

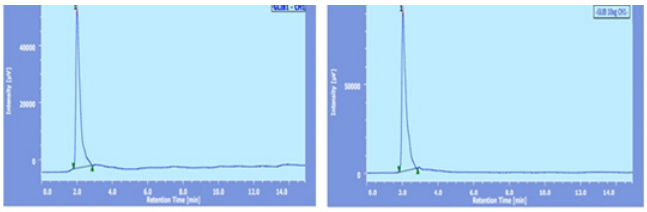
The stability of GBC in the mobile phase was investigated by keeping drug in the mobile phase for 24 hours. Turbidity was not found in the sample solution, indicating the stability of GBC in the selected mobile phase. The calibration curve was plotted in the concentration range of 5 to 50 ppm of GBC, and it was found to be linear with a correlation coefficient (R^2) of 0.999.

System Suitability Test

Calculating the percentage RSD of retention times, tailing factor, theoretical plates, and peak areas from five replicate injections allowed for the evaluation of the system appropriateness

Table 1: Statistical summary of system suitability parameters

Sr. No.	*Parameters*	*Mean*
1	Peak area	430924.4
2	Retention time	2.10
3	Capacity factor	0.10504
4	No. of theoretical plates	3346.71
5	Asymmetry	0.58742



A B

Figure 5: (A)Typical chromatogram of glibenclamide with retention time 2.1 min. of laboratory sample; (B) Typical chromatogram of Duoline® tablet formulation showing retention time 2.1 min

Table 2: Results of estimation of glibenclamide in marketed sample

Sr. No.	*Solution*	*Weight has taken in mg*	*Peak area*	*Mean % estimation ± S.D.*
1.	Standard	10	478936	-
2.	Sample	10	470389	99.37 ± 0.0083
		10	475218	
		10	483642	
		10	471486	
		10	490362	

parameters from standard chromatograms. The results are given in Table 1. The technique passed the system suitability test because all the requirements for system appropriateness were met.

Table 2 shows the results of the estimation of glibenclamide in a marketed sample using a standard reference. The analysis was conducted by measuring the peak areas of the standard and sample solutions. The "Mean %Estimation ± S.D." is 99.37 ± 0.0083, representing the mean percentage estimation of glibenclamide in the sample along with the standard deviation (Figure 5).

Validation Parameters

Accuracy by recovery study

The degree to which test results obtained by an analytical procedure was accurate in relation to their true value on the basis of recovery studies carried out using the suggested strategy accuracy was determined.

Recovery

The percentage of glibenclamide samples recovered ranges from 99.53 to 100.08%, and the mean recovery is 99.80%, indicating the method's high degree of accuracy. Table 3 displays the outcome.

Table 3: Statistical summary of validation parameters of glibenclamide

S. No.	*Parameter*	*Statistical data*	*Estimation of Glibenclamide*
1	Percentage drug recovery study	Mean	99.80
		±S.D.	0.002753
		R.S.D.	0.0027585
		C.V.	0.2758
2	Percentage drug recovery study (Intraday)	Mean	99.87
		±S.D.	0.005574
		R.S.D.	0.005822
		C.V.	0.582
3	Percentage drug recovery study (Interday)	Mean	99.82
		±S.D.	0.0058731
		R.S.D.	0.0058805
		C.V.	0.588
4	Different analyst	Mean	98.40
		±S.D.	0.013551
		R.S.D.	0.0013771
		C.V.	0.137

Precision

The assay for glibenclamide during the method's precision was determined to have a %R.S.D. of 0.0027585%, indicating good precision. Table 3 provides a summary of the findings.

Ruggedness

The intraday study was performed using a 10 µg/mL solution of GBC, and the peak area was measured after 3 hours. And the intraday investigation was carried out on days 1, 2, and 3. The RSD of 0.00137 showed that the process was reliable because it did not exhibit variance when applied by two individuals, according to the results of the various analyst examinations. Table 3 contains statistical information.

Linearity and range

Following the principles outlined in the ICH guidelines (GBC), the proposed method's linearity was evaluated. The analysis revealed that the method exhibited a linear concentration span encompassing 80 to 120% of the test concentration. The linear regression equation derived for GBC was determined as y = 44606x - 46783, accompanied by an R2 value of 0.998. In this context, 'x' symbolizes the concentration, 'y' denotes the observed response, and 'R2' represents the correlation coefficient. These results underscored a robust linear association between the peak area and the concentration of the analyte.

Acis hydrolysis

HPLC investigations of (GBC) subjected to diverse stress conditions have revealed distinct degradation patterns, as outlined in Table 4 and illustrated in Figure 6. The proposed methodology was employed to analyze all samples exposed to stress, facilitating the computation of the percentage of the drug that remained following degradation.

The procedure was carried out by refluxing (GBC) with 0.1 N HCl at 60°C for 3 hours. After 3 hours, the estimated

Table 4: Result of acid hydrolysis study of bulk and tablet

Study	*Sample*	*Conc. of reactant*	*Drug Conc.(*µg/mL*)*	*Exposure time and condition*	*Testing Interval (*hours*)*	*%Drug Remained By HPLC*
Acid Hydrolysis	Glibenclamide Bulk drug	0.1N HCl	10	3 hours reflux	0	99.56
					1	99.47
					2	97.40
					3	94.78
Acid Hydrolysis	Duoline® tablet	0.1N H.C.L.	10	3 hours reflux	0	99.36
					1	99.21
					2	98.55
					3	92.46

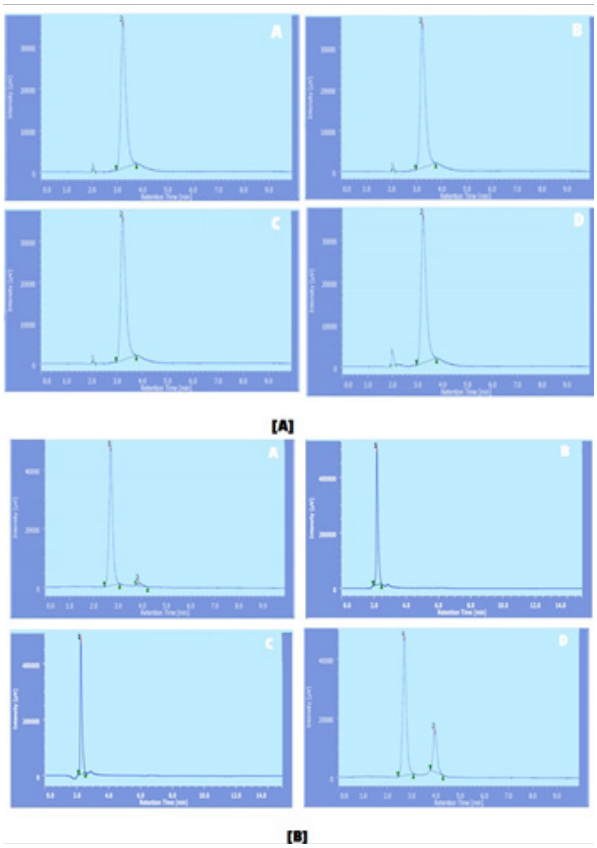


Figure 6: [A]Chromatogram after acid hydrolysis of bulk (A:0hr, B:1 hr, C:2hr, D:3hr) ; [B]: Chromatogram after acid hydrolysis of the tablet (A:0hr, B:1hr, C:2hr, D:3hr)

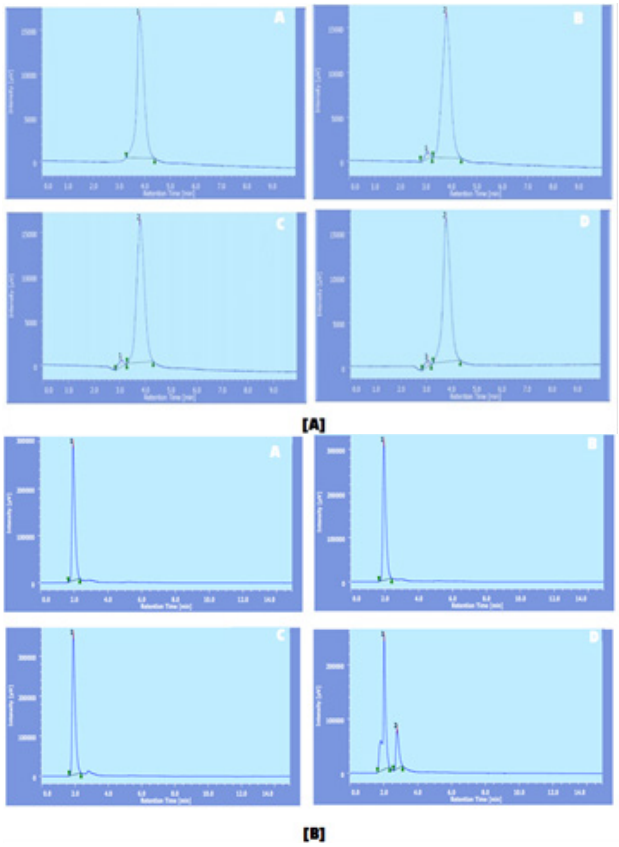


Figure 7: [A] Chromatogram after alkali hydrolysis of bulk (A:0hr, B:1 hr, C:2hr, D:3hr); [B]: Chromatogram after alkali hydrolysis of tablet (A:0hr, B:1 hr, C:2hr, D:3hr).

drug concentrations was 94.78% in bulk and 92.46% in tablets. According to this study, the drug's bulk stability in an acidic environment was only 5.22% lower than its tablet stability, which was 7.54% lower than its bulk stability under basic and oxidative conditions (Figure 6).

Alkali hydrolysis

GBC was refluxed with 0.1 N NaOH at 60ºC for 3 hours in its simplest form. After 3 hours, it was discovered that (GBC) was a little unstable in 0.1 N NaOH. study has left 99.87% of the bulk and 89.72% of the tablet formulation. As a result, GBC is more unstable in tablet formulation when exposed to basic conditions as opposed to acidic conditions shown in Table 5 and Figure 7.

Oxidation studies oxidative degradation of (GBC) was performed using hydrogen peroxide.

Hydrogen peroxide was refluxed on the GBC in an oxidative condition for three hours at 60ºC. After 3 hours, GBC was discovered to be more unstable under an oxidative condition than under an alkaline condition, with 84.49% of the bulk and 98.99% of the tablet formulation still present. in Table 6 and Figure 8 illustrates.

Table 5: Result of alkali hydrolysis study of bulk and tablet

Study	*Sample*	*Conc. of reactant*	*Drug Conc. (µg/mL)*	*Exposure time and condition*	*Testing Interval (*hours*)*	*%Drug Remained By HPLC*
Alkali Hydrolysis	Glibenclamide Bulk drug	0.1N NaOH	10	3 hours reflux	0	99.56
					1	98.23
					2	97.19
					3	94.87
Acid Hydrolysis	Duoline® tablet	0.1N NaOH	10	3 hours reflux	0	99.45
					1	99.37
					2	98.05

Table 6: Result of peroxide study of bulk and tablet

Study	*Sample*	*Conc. of reactant*	*Drug Conc. (µg/mL)*	*Exposure time and condition*	*Testing Interval (*hours*)*	*%Drug Remained By HPLC*
Oxidation Study	Glibenclamide Bulk drug	3% Hydrogen peroxide	10	3 hours reflux	0	98.75
					1	93.02
					2	90.78
					3	84.49
Oxidation study	Duoline® tablet	3% Hydrogen Peroxide	10	3 hours reflux	0	99.93
					1	99.67
					2	99.31
					3	98.99

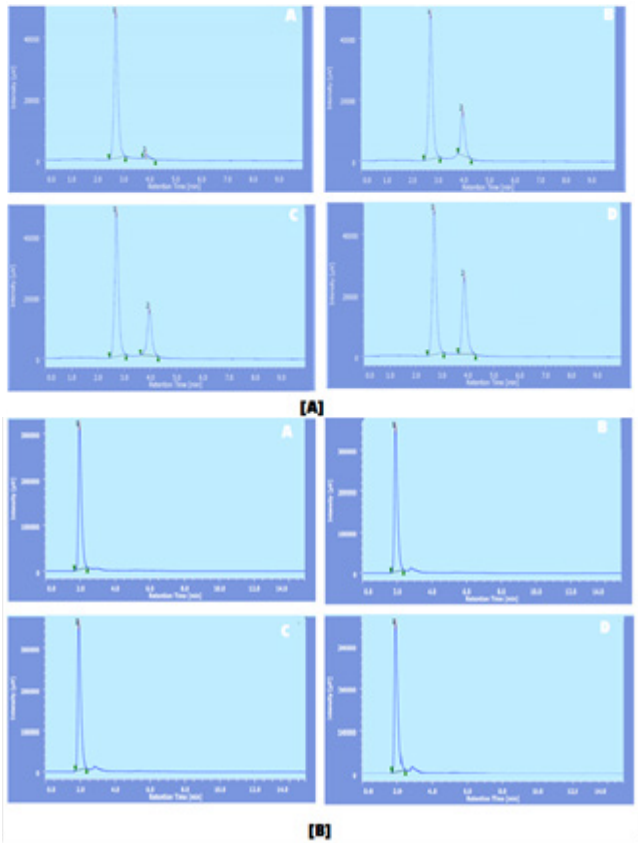


Figure 8: [A] Chromatogram after peroxide study of bulk (A:0hr, B:1 hr, C:2hr, D:3hr); [B]: Chromatogram after peroxide study of tablet (A:0hr, B:1 hr, C:2hr, D:3hr).

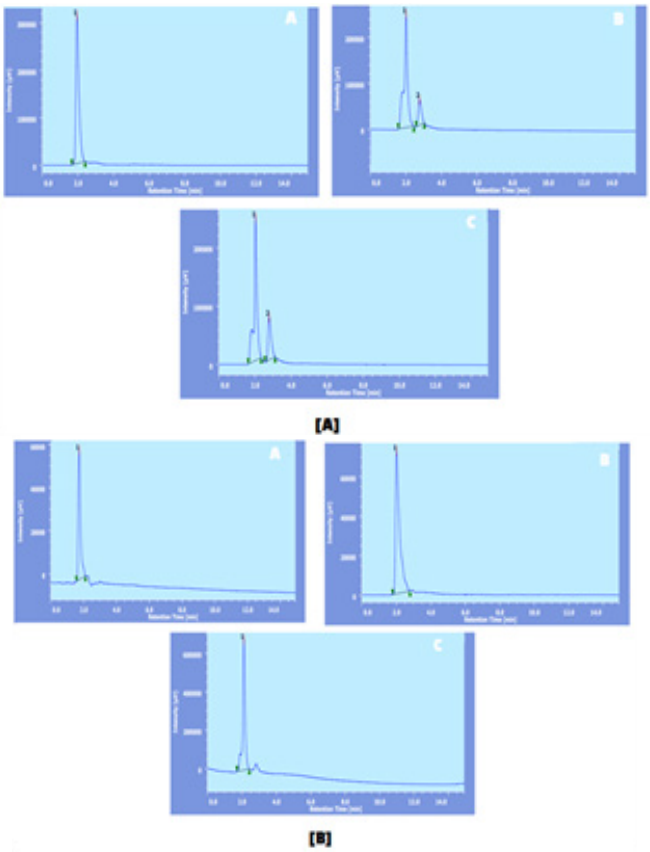


Figure 9: [A] Chromatogram after photolytic study of bulk A:0 hr, B:8 hr and C:12 hr; [B] Chromatogram after photolytic study of tablet A:0 hr, B:8 hr and C:12 hr

Table 7: Result of Photolytic degradation of bulk and tablet

Study	*Sample*	*Drug Conc. (µg/mL)*	*Exposure time and condition*	*Testing Interval (hour)*	*%drug Remained By HPLC*
Photolytic Degradation	Glibenclamide Bulk drug	10	Kept drug In U.V. light for 12 hours	0	99.73
				8	89.65
				12	88.37
Photolytic Degradation	Duoline® tablet	10	Kept drug In U.V. light for 12 hours	0	98.48
				8	98.36
				12	96.97

Table 8: Result of thermal study of bulk and tablet

Study	*Sample*	*Drug Conc. (µg/mL)*	*Exposure time and condition*	*Testing Interval (hours)*	*%drug Remained By HPLC*
Thermal Degradation	Glibenclamide Bulk drug	10	Kept drug In oven For 12 hours at 50 ºC	0	97.84
				8	95.35
				12	87.42
Thermal Degradation	Duoline® tablet	10	Kept drug In oven For 12 hours at 50 ºC	0	99.54
				8	99.28
				12	94.84

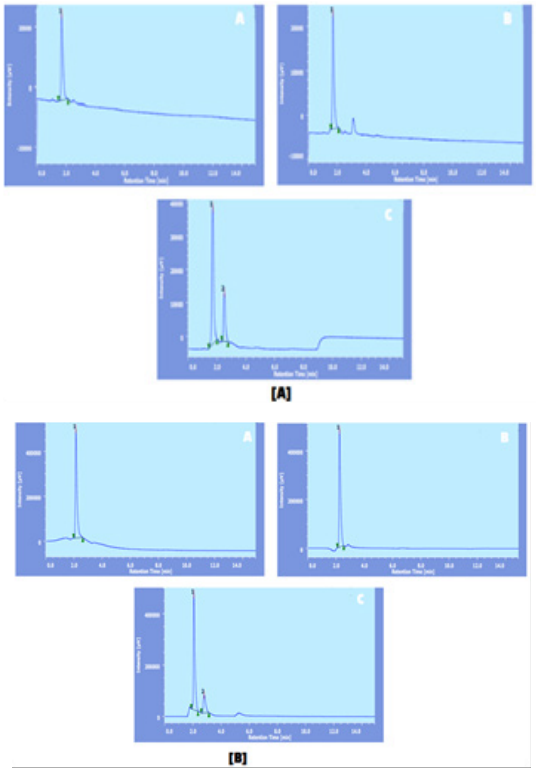


Figure 10: [A] Chromatogram after thermal study of bulk A:0 hr, B:8 hr and C:12 hr; [B]; Chromatogram after thermal study of tablet A=(0 hr), B=(8 hr), and C=(12 hr)

Photolytic degradation (UV Light)

The GBC. was spread on a petri plate and placed inside the photostability chamber under the UV light for 12 hours. 88.37% in bulk and 96.97% of tablet formulation remained after 12 hours; this shows that the drug was more stable in tablet formulation as compared to bulk in photolytic conditions. Shown in Table 7 and Figure 9.

Thermal stability Studies (Dry heat)

The drug was spread on a Petri plate and placed in an oven at 50°C for 12 hours and after 12 hours, 87.42% in bulk and 94.84% in tablet formulation remained. This shows that the drug was less prone to thermal degradation in tablet formulation as compared to bulk shown in Table 8 and Figure 10.

CONCLUSION

The work aimed to design and validate a stability-indicating RP-HPLC technique for glibenclamide (GBC). A stability-indicating assay technique was developed to investigate the degradation profile of GBC.

GBC is more stable in acidic and basic circumstances than in oxidative, photolytic, or thermal settings. As a result, it is more sensitive to oxidative, photolytic, and thermal deterioration. In acidic, basic, and oxidative environments, the tablet formulation was shown to be more stable We could not perform photolytic and thermal degradation experiments for three months as required by ICH standards because of the pandemic restrictions; therefore, we conducted this research for 12 hours. The sample preparation technique is economical, using a mobile phase with a basic composition that extends the column's life. The values obtained from RP-HPLC investigations for degradation products assist us in confirming the existence of the degradants. The degradation tests conducted according to ICH rules demonstrated that the approach chosen is stable. The method developed for estimating GBC was highly sensitive, accurate, and reproducible. GBC in bulk and pharmaceutical dosage forms may be routinely analyzed using the developed stability-indicating technique.

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