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VALIDATION OF A FORCED DEGRADATION UPLC METHOD FOR ESTIMATION OF GLIBENCLAMIDE IN ORAL DOSAGE FORM

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ABSTRACT

A selective, precise, accurate and stability indicating UPLC method is validated for estimation of Glibenclamide in oral dosage form. The method employed, with Hypersil C18 (100 mm x 2.1 mm, 1.7 µm) column in gradient mode, with mobile phase of Methanol and Acetonitrile in the ratio of 80:20 %v/v. The flow rate was 1.2 ml/min and effluent was monitored at 272nm. Retention time was found to be 8.623±0.11 min. The method was validated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 2- 10µg/ml respectively. The LOD and LOQ values for were found to be 0.19543(µg/ml) and 0.59223 (µg/ml) respectively. No chromatographic interference from excipients and degradants were found. The proposed method was successfully used for estimation of Glibenclamide in oral dosage form.

KEYWORDS: Glibenclamide, oral dosage form, stability indicating UPLC method.

INTRODUCTION

Glibenclamide compound belongs to the class of organic compounds known as benzenesulfonamides. These are organic compounds containing a sulfonamide group that is S-linked to a benzene ring.

Category: Sulfonylurea Compounds.

Glibenclamide

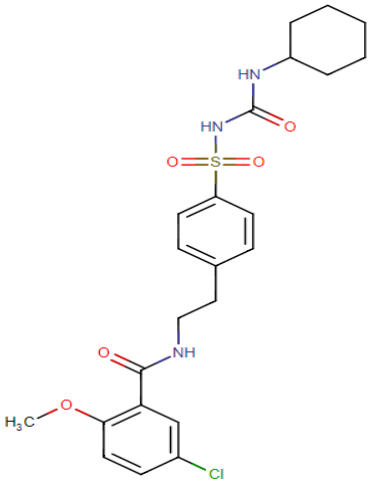


Fig. 1: Structure of Glibenclamide.

Nomenclature: 5-chloro-N-[2-(4 {[(cyclohexylcarbamoyl) amino] sulfonyl} phenyl) ethyl]-2-methoxybenzamide

Molecular formula: $C_{23}H_{28}ClN_3O_5S$
Molecular weight: 494.004

Solubility: Water solubility: 4 mg/L (at 25 °C), pKa (Strongest Acidic) 4.32, pKa (Strongest Basic) - 1.2

Mechanism of action
Sulfonylureas such as Glibenclamide bind to ATP-sensitive potassium channels on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the membrane. Depolarization stimulates calcium ion influx through voltage-sensitive calcium channels, raising intracellular concentrations of calcium ions, which induces the secretion, or exocytosis, of insulin.

Half life: 1.4-1.8 hours.
Generic Name: Glibenclamide
Brand Names: Daonil

Validation of Analytical Methods (USP/ICH)
Method validation, according to the United States Pharmacopeia (USP), is performed to ensure that an analytical methodology is accurate, specific,

reproducible, and rugged over the specified range that an analyte will be analyzed. Regulated laboratories must perform method validation in order to be in compliance with FDA regulations. In a 1987 guideline (Guideline for Submitting Samples and Analytical Data for Methods Validation), the FDA designated the specifications in the current edition of the USP as those legally recognized when determining compliance with the Federal Food, Drug and Cosmetic Act can be referred to as the "eight steps of method validation"

Experimental
Materials

Equipments	Source
Ultra Pressure Liquid Chromatography (UPLC)	Acquity UPLC Systems, Waters Laboratories
Electrospray ionization and MS-MS	Mass Spectrometer PE Sciex Model: API 3000
Chromatographic data software	Empower
Column	C18 column (250 ×4.6 mm id)—ACE Generix
Detector	PDA
Injector	Automated
Electronic Balance	Eagle
Sonicator	Band Line Sonerex
p^H Meter	Lab India p^H meter

METHODOLOGY

Method Validation
The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc. The described method extensively validated in terms of specificity, system suitability, linearity, accuracy, precision, limit of detection, limit of quantification and robustness.

Forced degradation studies of our selected pharmaceutical drugs.
In order to establish the analytical method for a stability indicating method, the drugs are subjected to various stress conditions to conduct forced degradation studies. Stress studies were carried out under the conditions of acid/base hydrolysis, oxidation, reduction, in accordance with ICH Q1A (R2). Several trials with different severity of each stressed condition are to be conducted, so that upto 10-30% degradation is to be achieved.

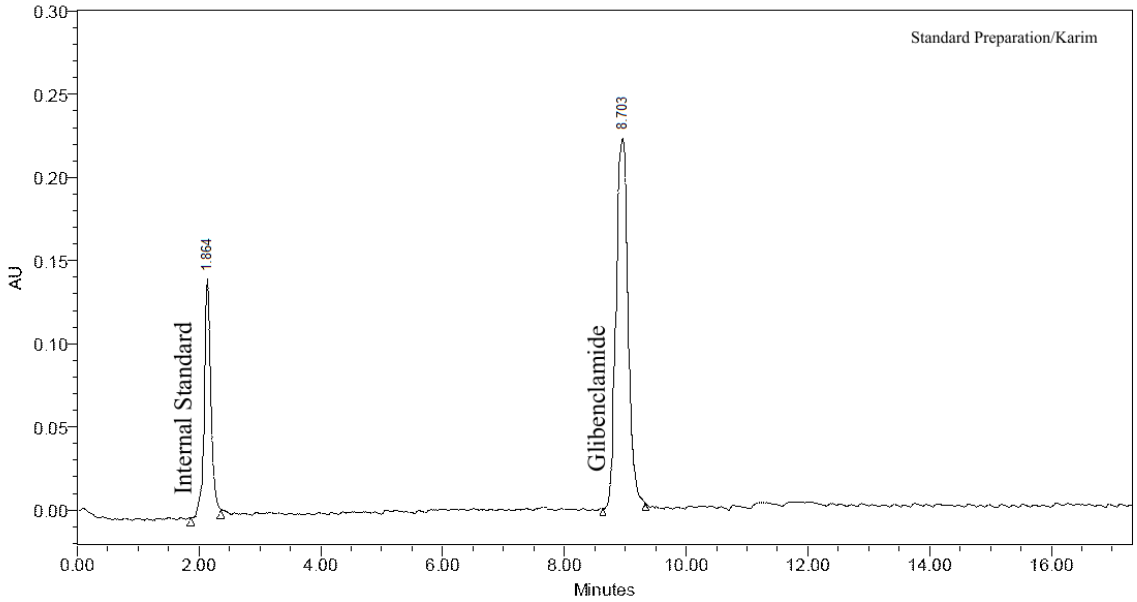
RESULTS

Glibenclamide
Preparation of Standard Stock Solution
Preparation of Diluent
In order to achieve the separation under the optimized conditions after experimental trials that can be summarized. Stationary phase like Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm) column was most suitable one, since it produced symmetrical peaks with high resolution and a very good sensitivity and with good resolution. The flow rate was maintained 1 mL min-1 shows good resolution. The PDA detector response of Glibenclamide was studied and the best wavelength was found to be 230 nm showing highest sensitivity.

The mixture of two solutions Chloroform and Acetonitrile in the ratio of 60:40 %v/v" with gradient programming was used as mobile phase at 1 mL/min was found to be an appropriate mobile phase for separation of Glibenclamide. The column was maintained at ambient temperature.

Preparation of internal standard solution
Weighed accurately about 10 mg of D-Phenylalanine working standard and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter.

Preparation of Glibenclamide standard solution
Weighed accurately about 10 mg of Glibenclamide and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter.



Chromatogram of standard preparation of Glibenclamide.

Accuracy

Table 1: Results of Accuracy Study (Glibenclamide).

Glibenclamide						
Level %	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Mean Recovery (%)**	**Std.Dev**	**% RSD**
50	02.06	02.05	99.51	99.28	0.3897	0.39%
100	04.12	04.10	99.50			
150	06.18	06.17	98.83			

System Precision

Procedure

The parameters, retention time (RT), theoretical plates (N), tailing factor (T), peak asymmetry (As) and repeatability were evaluated at a concentration of 4µg/mL (Glibenclamide).

Table 2: Results of System Precision (Glibenclamide).

Parameters	**Glibenclamide**
Retention time (min) ± % RSD	8.775 ± 0.04
Theoretical plates ± % RSD	2377.56 ± 0.50
Asymmetry ± % RSD	1.08 ± 0.05
Repeatability (% RSD)	0.11

Table 3 Results of Method Precision (Glibenclamide)

Replicate		***Glibenclamide***	
S.No.	**Concentration Taken (µg/ml)**	**Area**	**%LC**
1	04.00	26138	99.99%
2		26141	99.98%
3		26139	99.98%
4		26134	99.99%
5		26140	99.98%
6		26142	99.97%
Average			99.98%
Std.Dev			0.00752
% RSD			0.01%
Standard weight			4mcg
Standard potency			99.80%

Linearity

Procedure: The linearity of the method was determined at five concentration levels ranging from 2-10 µg/mL for Glibenclamide."

Table 4: Result of Linearity Studies (Glibenclamide).

Glibenclamide		
Linearity level	**Concentration in µg/mL**	**Area**
1	2 µg/mL	20149
2	4 µg/mL	26136
3	6 µg/mL	34182
4	8 µg/mL	41433
5	10 µg/mL	46134
Correlation co-efficient	0.9968	
Slope	3363.35	
Intercept	13426.7	

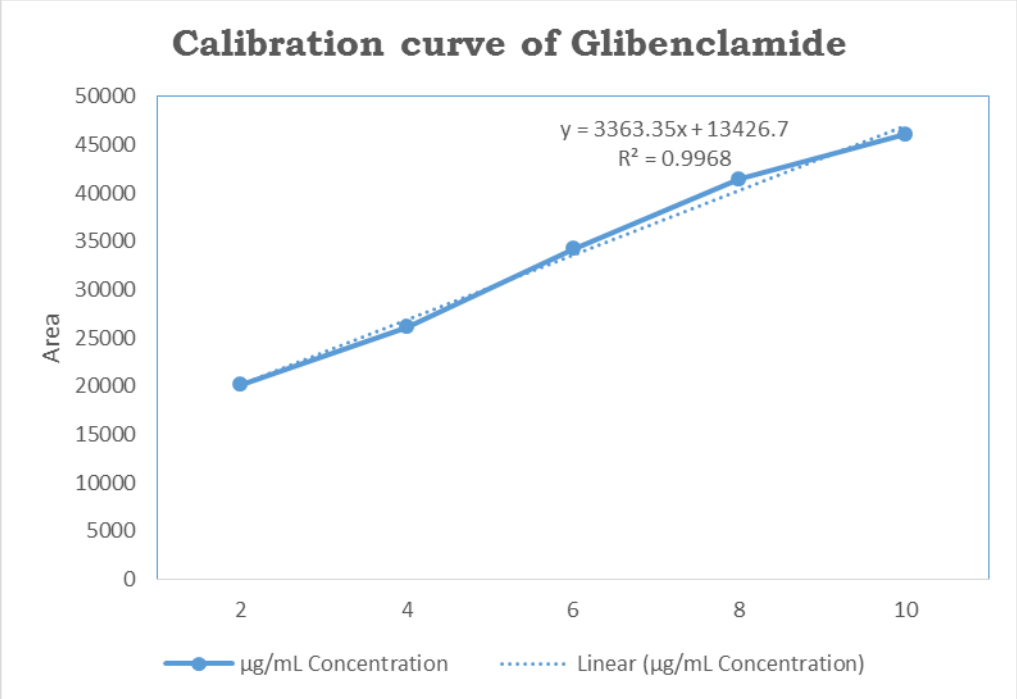


Fig. 1: Calibration curve of Glibenclamide.

Robustness

Table 5: Result of Robustness Studies (Glibenclamide).

Robustness Studies			
Parameter	**Value**	**Peak Area**	**% RSD**
Flow Rate	**Low**	27548	0.13%
	Actual	27592	
	Plus	27619	
Temperature	**Low**	27563	0.16%
	Actual	27646	
	Plus	27629	
Wavelength	**Low**	27548	0.14%
	Actual	27593	
	Plus	27626	

Ruggedness

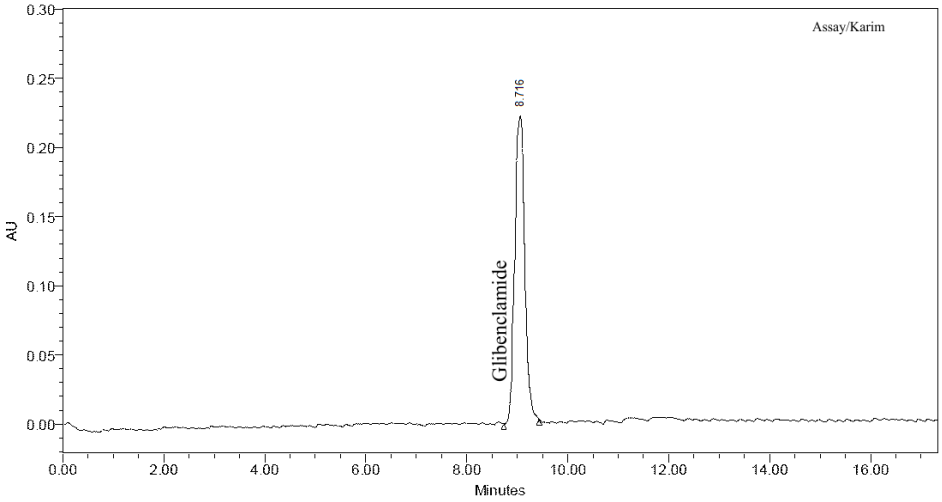
Table 6: Result of Ruggedness Studies (Glibenclamide.

Glibenclamide			
Ruggedness			
Parameter	**Peak Area**	**% RSD**	**%LC**
Intraday precision	27158	0.11%	99.77%
	27212		99.97%
	27164		99.79%
Inter day precision	27145	0.15%	98.72%
	27229		99.96%
	27182		99.86%
Instrument:1 Acquity UPLC Waters,2695H	27118	0.04%	99.62%
	27139		99.70%
	27121		99.63%
Instrument:2 Agilent Technologies,1290	27117	0.04%	99.62%
	27134		99.68%
	27117		99.62%
Average			**99.66**
Std. Dev			**0.322**
%RSD			**0.32%**

Forced Degradation Studies

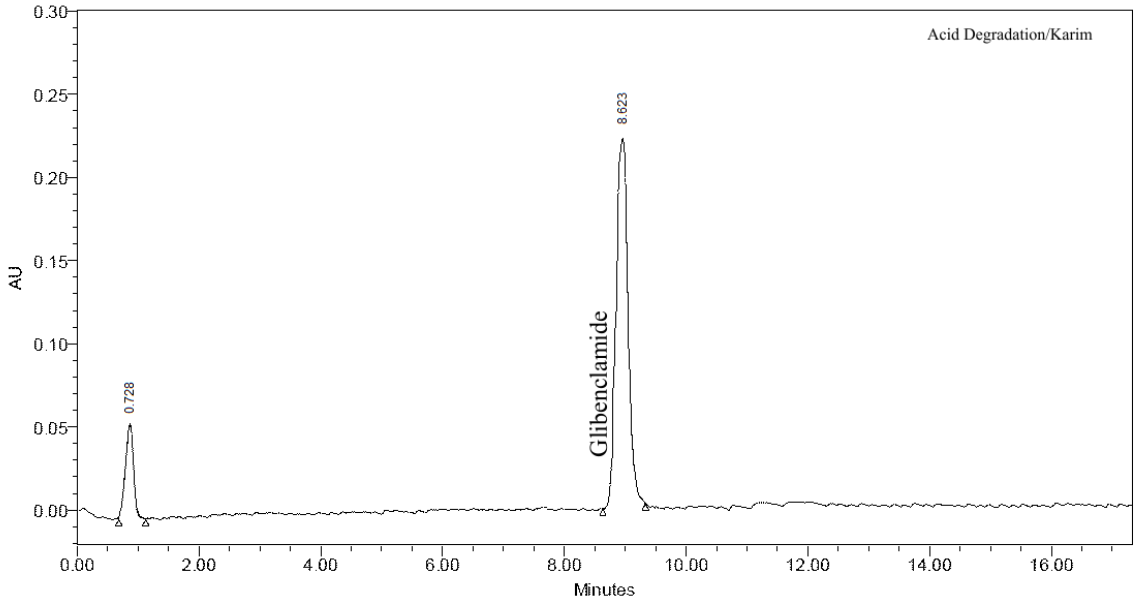
Sample Control: An accurate 10 ml of the prepared pure drug stock solution of working standard was transferred to a clean and dry RBF. The volume of the sample was 100µg/ml. It was injected into the UPLC system against a blank of Methanol and Acetonitrile in the ratio of 80:20 %v/v after optimizing the mobile phase composition, chromatogram was recorded.

Assay of Glibenclamide (Sample Control)



a. Acidic Degradation

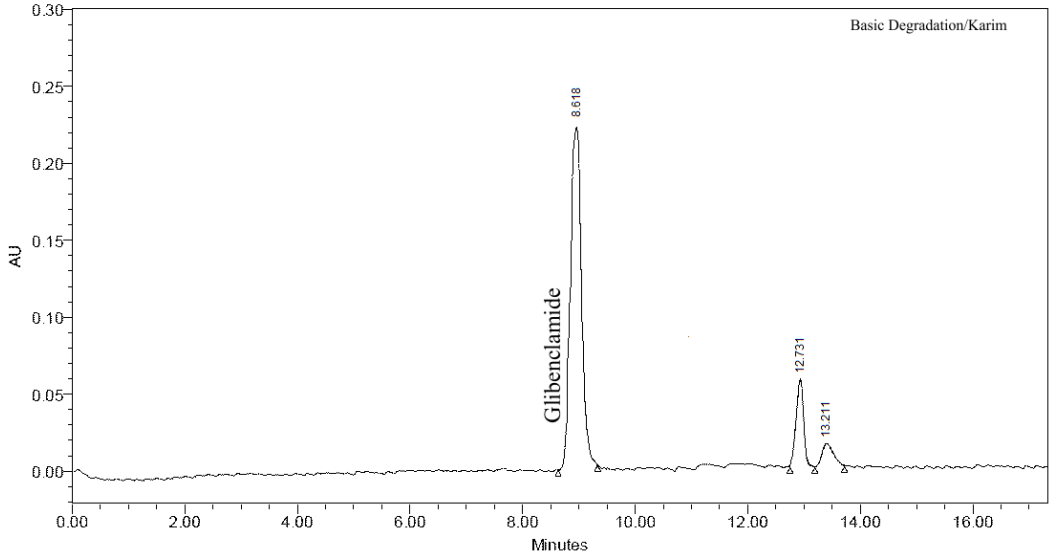
An accurate 10 ml of pure drug sample solution was transferred to a clean and dry round bottom flask (RBF). 30 ml of 0.1 N HCl was added to it. It was refluxed in a water bath at 60°C for 4 hours. Drug became soluble after reflux which was insoluble initially. Allowed to cool at room temperature. The sample was then neutralized using 2N NaOH solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of Methanol and Acetonitrile in the ratio of 80:20 %v/v after optimizing the mobile phase composition, chromatogram was recorded and shown in Chromatogram."



Chromatogram showing the degraded products in Acidic degradation

b. Basic Degradation

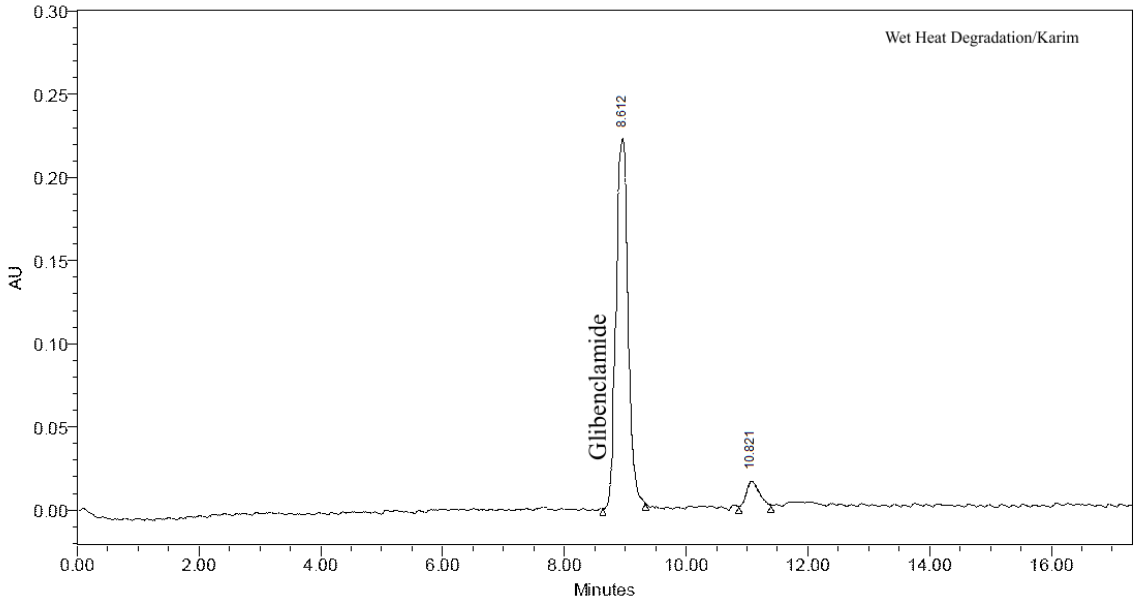
An accurate 10 ml of pure drug sample solution was transferred to a clean and dry RBF. 30 ml of 0.1N NaOH was added to it. It was refluxed in a water bath at 60°C for 4 hours. Drug became soluble after reflux which was insoluble initially. It was allowed to cool at room temperature. The sample was then neutralized using 2N HCl solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of Methanol and Acetonitrile in the ratio of 80:20 %v/v after optimizing the mobile phase composition, chromatogram was recorded and shown in Chromatogram."



Chromatogram showing the degraded products in Basic degradation

c. Wet heat degradation

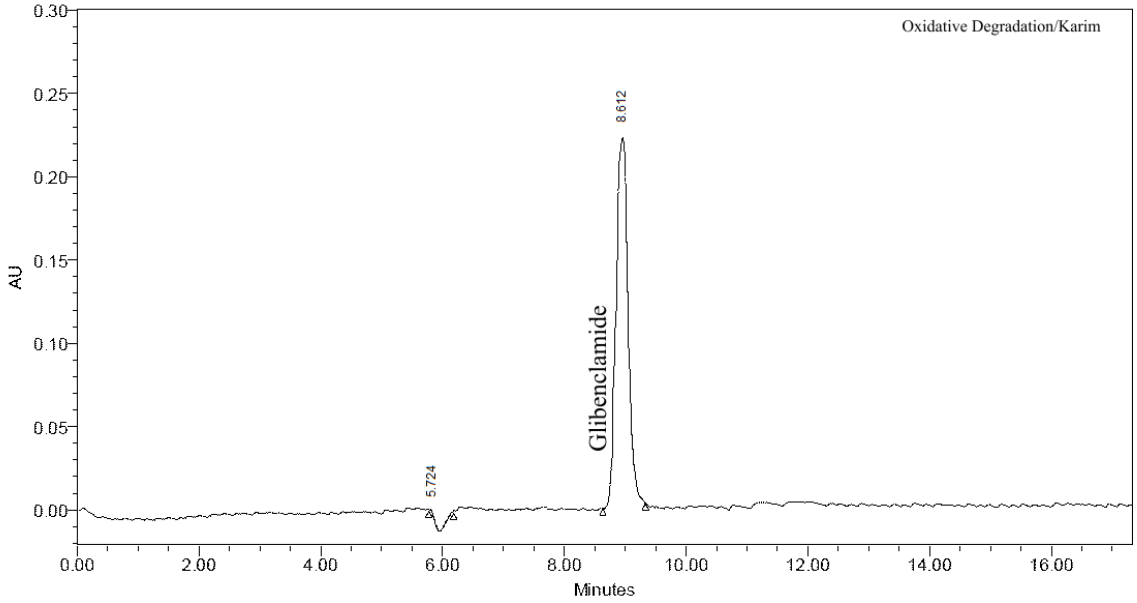
Accurate 10 ml of pure drug sample was transferred to a clean and dry RBF. 30 ml of HPLC grade water was added to it. Then, it was refluxed in a water bath at 60°C for 6 hours uninterruptedly. After the completion of reflux, the drug became soluble and the mixture of drug and water was allowed to cool at room temperature. Final volume was made up to 100 ml with HPLC grade water to prepare 100 ppm solution. It was injected into the UPLC system against a blank of Methanol and Acetonitrile in the ratio of 80:20 %v/v after optimizing the mobile phase composition, chromatogram was recorded and shown in Chromatogram."



Chromatogram showing the degraded products in Wet heat degradation

d. Oxidation with (3%) H_2O_2

Approximately 10 ml of pure drug sample was transferred in a clean and dry 100 ml volumetric flask. 30 ml of 3% H_2O_2 and a little methanol was added to it to make it soluble and then kept as such in dark for 24 hours. Final volume was made up to 100 ml using water to prepare 100 ppm solution. The above sample was injected into the UPLC system. The chromatogram was recorded and shown in Chromatogram.



Chromatogram showing the degraded products in Oxidative degradation

Table 7: Summary of Forced Degradation Studies (Glibenclamide).

Nature of Stress	Degradation condition	Time(h)	Number of degradation products (Rt)
Acidic	60°C	3	1 (0.728)
Basic	60°C	9	2 (12.731, 13.211)
Oxidative	RT	48	1 (5.724)
Wet Heat	105°C	24	1 (10.821)

EVALUATION OF METHODS

Forced Degradation Studies

- **Analysis of Glibenclamide**

Table 8: Results of Forced Degradation Assays (Glibenclamide).

Conditions	Sample Amount (µg/ml)	Peak Area	% claim	% Degradation
Sample Control	04.15	26139	91.69%	-
Acidic Degradation	04.08	24721	*86.73%*	4.96%
Basic Degradation	04.05	23581	*82.72%*	8.97%
Oxidative Degradation	04.03	24357	*85.44%*	6.25%
Wet Heat	04.06	25832	*90.62%*	1.07%

Calculation formula for Glibenclamide

$$\%\ Assay = \frac{AT}{AS} \times \frac{W1}{100} \times \frac{1}{25} \times \frac{100}{W2} \times \frac{25}{1} \times \frac{AW}{LC} \times P$$

Whereas,"
AT = Average area of test preparation, 26139"
AS = Average area of standard preparation, 28358"
W1 = Weight taken of reference standard (µg), 04.15"
W2 = Weight taken of test sample (µg), 04.25"
AW = Average weight of sample (µg), 3057"
LC = Label claim (µg), 3000"
P = Potency of reference standard (%), 99.98%"

$$\%\ Assay = \frac{AT}{AS} \times \frac{W1}{100} \times \frac{1}{25} \times \frac{100}{W2} \times \frac{25}{1} \times \frac{AW}{LC} \times P$$

Sample Control (Glibenclamide)

$$\%\ Assay = \frac{26139}{28358} \times \frac{04.15}{100} \times \frac{1}{25} \times \frac{100}{04.25} \times \frac{25}{1} \times \textbf{Error!} \times 99.98$$

= 91.69%

Acidic Degradation (Glibenclamide)

$$\%\ Assay = \frac{24721}{28358} \times \frac{04.15}{100} \times \frac{1}{25} \times \frac{100}{04.25} \times \frac{25}{1} \times \textbf{Error!} \times 99.98$$

= 86.73%

Basic Degradation (Glibenclamide)

$$\%\ Assay = \frac{23581}{28358} \times \frac{04.15}{100} \times \frac{1}{25} \times \frac{100}{04.25} \times \frac{25}{1} \times \textbf{Error!} \times 99.98$$

= 82.72%

Oxidative Degradation (Glibenclamide)

$$\%\ Assay = \frac{24357}{28358} \times \frac{04.15}{100} \times \frac{1}{25} \times \frac{100}{04.25} \times \frac{25}{1} \times \textbf{Error!} \times 99.98$$

= 85.44%

Wet Heat (Glibenclamide)

$$\%\ Assay = \frac{25832}{28358} \times \frac{04.15}{100} \times \frac{1}{25} \times \frac{100}{04.25} \times \frac{25}{1} \times \textbf{Error!} \times 99.98$$

= 90.62%

CONCLUSION

A selective, precise, accurate and stability indicating UPLC method is validated for estimation of Glibenclamide in oral dosage form. The method employed, with Hypersil C18 (100 mm x 2.1 mm, 1.7 µm) column in gradient mode, with mobile phase of Methanol and Acetonitrile in the ratio of 80:20 %v/v. The flow rate was 1.2 ml/min and effluent was monitored at 272nm. Retention time was found to be 8.623±0.11 min. The method was validated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 2- 10µg/ml respectively. The LOD and LOQ values for were found to be 0.19543(µg/ml) and 0.59223 (µg/ml) respectively. No chromatographic interference from excipients and degradants were found. The proposed method was successfully used for estimation of Glibenclamide in oral dosage form.

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