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ESTIMATION OF GLIBENCLAMIDE PRESENT IN TABLETS BY RP-HPLC
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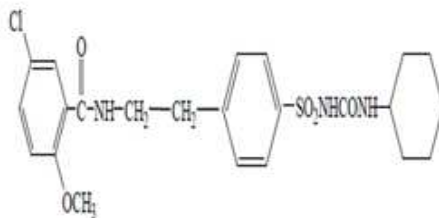
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ABSTRACT:

A simple, sensitive, precise and stability indicating Reverse phase high performance liquid chromatographic method has been developed for the quantitative analysis of drug Glibenclamide present in tablet formulation and bulk drug. The HPLC separation was achieved on Symmetry C18 (4.6 x 100mm, 5 μ m, Make: XTerra) with the mobile phase and detection at 228nm. The proposed method provided linear responses within the concentration range 10-50 μ g mL⁻¹ for Glibenclamide. LOD and LOQ values for the active substance were 0.03 and 0.07 mg mL⁻¹, respectively. Correlation coefficients (*r*) of the regression equations for the impurities were greater than 0.999 in all cases. The precision of the method was demonstrated using intra- day assay RSD% values which were less than 1% in all instances. No interference from any components of pharmaceutical dosage forms or degradation products was observed.

Key Words: Working standard of Glibenclamide, Reverse Phase High Performance Liquid Chromatography, and validation.

Introduction:



Glibenclamide, *N*-(4-[*N* (cyclohexylcarbamoyl)sulfamoyl] phenethyl)-2-methoxybenzamide or 1-[[p-[2-(5-chloro-o-anisamido)ethyl]phenyl]-sulphonyl-3cyclohyxylurea, a sulphonyl urea derivative ^(1,2), a white crystalline odorless powder without any taste, practically insoluble in water, sparingly soluble in methylene chloride, slightly soluble in ethanol, methanol and insoluble in in diethyl ether .It dissolves in dilute alkali hydroxides⁽³⁻⁶⁾ .Glibenclamide is an anti diabetic drug which belongs to the second generation oral hypoglycemic class. It is used to assist in the control of mild to moderately severe type 2 diabetes mellitus that does not require any insulin, a drug of choice for initiating treatment in noninsulin-dependent diabetes when diet and weight control fails ^(7, 8). A high performance liquid chromatographic method is described for estimation of Glibenclamide in human serum⁹, Simultaneous Spectrophotometric Estimation and Validation of Three Component Tablet Formulation Containing Pioglitazone Hydrochloride, Metformin Hydrochloride and Glibenclamide ¹⁰ ,Spectrophotometric Estimation of Glibenclamide ^(11,12) , HPLC method was developed for the simultaneous determination of metformin and Glibenclamide^(13,14) and Reversed-phase high performance liquid chromatography (RP-HPLC) method was developed for screening counterfeit medicines¹⁵ were developed. The present method was rapid, accurate, précised and economic method for routine analysis of Glibenclamide present in tablets.

Reagents and Standard – Glibenclamide tablets:

Water HPLC Grade, Glibenclamide Working Standard, and Acetonitrile and Potassium dihydrogen phosphate.

Chromatographic Parameters

Equipment	: High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector.
Column	: Symmetry C18 (4.6 x 100mm, 5 µm, Make: XTerra) or equivalent
Flow rate	: 0.8 mL per min
Wavelength	: 228 nm
Injection volume	: 20 µl
Column oven	: Ambient
Run time	: 5 min

Preparation of Phosphate buffer: Weigh 7.0 grams of Potassium dihydrogen Phosphate into a 1000ml beaker, dissolve and diluted to 1000ml with HPLC water. Adjusted the pH to 7.0 with Potassium Hydroxide.

Preparation of mobile phase: Mix a mixture of above buffer 600mL (60%) and 400 mL of Methanol HPLC (40%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 μ filter under vacuum filtration.

Diluent Preparation: Mobile Phase as diluent.

Preparation of the Glibenclamide Standard & Sample Solution:

Standard Solution Preparation: Accurately weigh and transfer 10mg of Glibenclamide Working standard into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 5 ml of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluents. Mix well and filter through 0.45 μ m filter. Further pipette 3 ml of the above stock solution into a 10ml volumetric flask and dilute upto the mark with diluents. Mix well and filter through 0.45 μ m filter

Sample Solution Preparation: Weigh 5 Glibenclamide Tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of Glibenclamide into a 10 mL volumetric flask. Add about 7 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 μ m filter.

Further pipette 5 ml of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 μ m filter. Further pipette 3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 μ m filter

Procedure:

Inject 20 μ L of the standard, sample into the chromatographic system and measure the area for the Glibenclamide peak and calculate the % Assay by using the formulae.

System Suitability:

Tailing factor for the peak due to Glibenclamide in Standard solution should not be more than 1.5. Theoretical plates for the Glibenclamide peak in Standard solution should not be less than 2000

Precision:

Accurately weigh and transfer 10 mg of Glibenclamide Working standard into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 5 ml of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 μ m filter. Further pipette 3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 μ m filter.

Intermediate Precision/Ruggedness:

Accurately weigh and transfer 10 mg of Glibenclamide Working standard into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 5 ml of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 μ m filter. Further pipette 3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 μ m filter.

Accuracy:

Accurately weigh and transfer 10mg of Glibenclamide Working standard into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 5 ml of the above stock solution into a 50ml volumetric flask and dilute to the mark with diluent. Mix well and filter through 0.45 μ m filter. Further pipette 3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 μ m filter

Linearity:

Accurately weigh and transfer 10mg of Glibenclamide API sample into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 5 ml of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Limit of Detection:

Preparation of 30µg/ml solution:

Accurately weigh and transfer 10mg of Glibenclamide Working standard into 10mL volumetric flasks add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 5 ml of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Preparation of 0.8% solution At Specification level (0.024µg/ml solution):

Pipette 1mL of 10µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. Pipette 1mL of 10µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Further pipette 0.8mL of above diluted solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Limit of quantification:

Preparation of 30µg/ml solution:

Accurately weigh and transfer 10mg of Glibenclamide Working standard into a 10 mL Volumetric flasks add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 5 ml of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette 3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter

Preparation of 2.5% solution At Specification level (0.07µg/ml solution):

Pipette 1mL of 10µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. Pipette 1mL of 10µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. Further pipette 2.5mL of above diluted solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

A). the flow rate was varied at 0.6 to 1.0 ml/min: Standard solution 30 µg/ml was prepared and

Analysed using the varied flow rates along with method flow rate.

B). The Organic composition in the Mobile phase was varied from 35% to 45%:

Standard solution 30 µg/ml was prepared and analysed using the varied Mobile phase

Composition along with the actual mobile phase composition in the method

Calculation:

$$\text{Assay \%} = \frac{\text{AT}}{\text{AS}} \times \frac{\text{WS}}{\text{DS}} \times \frac{\text{DT}'}{\text{WT}} \times \frac{\text{P}}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:

AT = Peak Area of Glibenclamide obtained with test preparation

AS = Peak Area of Glibenclamide 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

Results and Discussions:

System suitability;

Standard solution is injected five times and Flow rate was maintained at 0.8 ml/min. temperature of column kept ambient and the column effluents were monitored at 228 nm chromatograms were taken and System suitability parameters were computed. The system suitability was calculated as per ICH guidelines (figure no.2, 3).

Figure No-2: Glibenclamide sample.

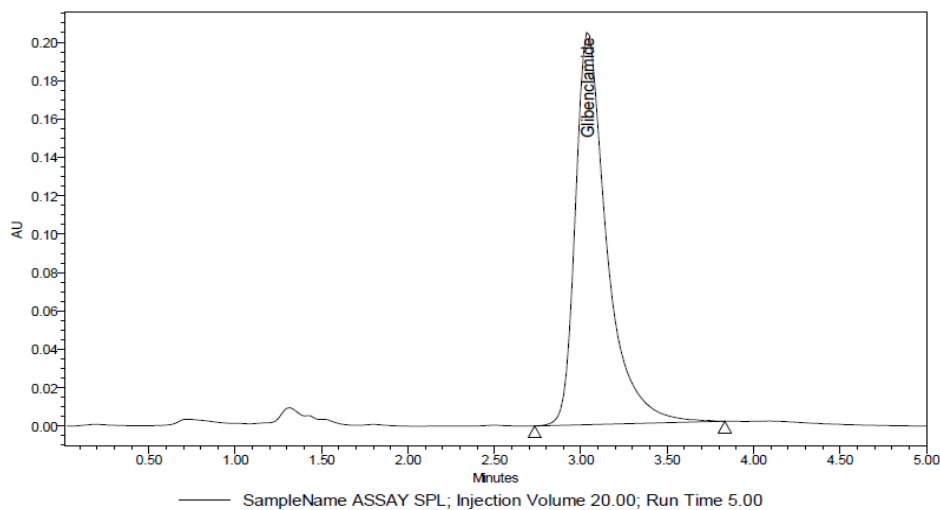
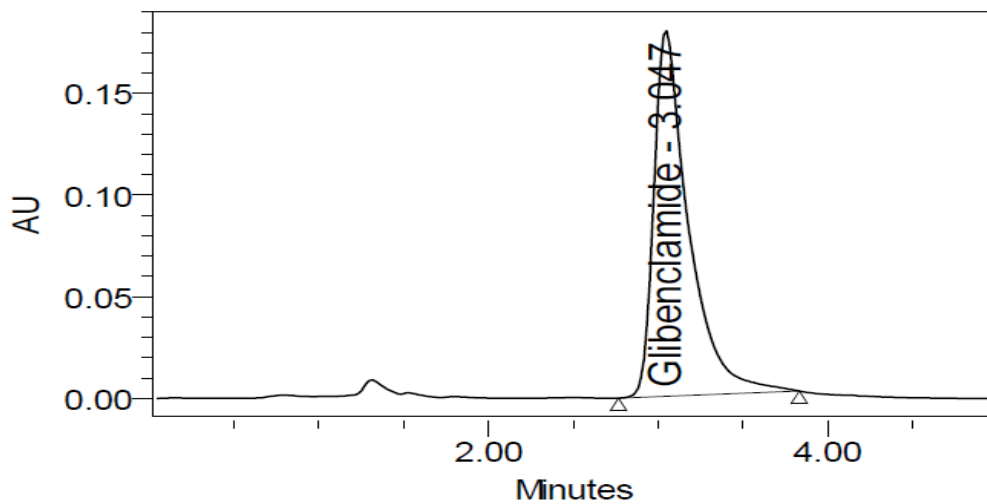


Figure No-3: Glibenclamide standard.



Precision:

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample preparation was carried out in same manner as described in sample preparation. Percentage relative standard deviation (percentage RSD) was found to be less than 2% that proves method is precise (table no.1).

Table No-1: Summary of precision:

Injection	Area
Injection-1	2587374
Injection-2	2585151
Injection-3	2596144
Injection-4	2587725
Injection-5	2576851
Average	2586649
Standard Deviation	6893.7
%RSD	0.27

Intermediate precision:

The intermediate precision of method carried out by using the different column with same dimensions on different day and area percentage RSD found to be 0.42 (table no.2).

Table No-2: Summary of Intermediate Precision

Injection	Area
Injection-1	2540013
Injection-2	2539567
Injection-3	2526321
Injection-4	2546914
Injection-5	2555425
Average	2541648
Standard Deviation	10717.5
%RSD	0.42

Accuracy (Recovery studies):

To check the degree of accuracy of the method, recovery studies were performed in triplet by standard addition method at 50%, 100% and 150% concentration levels. Known amounts of standard Glibenclamide was added to the pre-analyzed samples and subjected to the proposed HPLC method. Results of recovery studies are shown in table no.3.

Table No-3: Summary of Recovery studies.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	14753 40	5.68	5.78	98.2%	99.8%
100%	25449 55	10.0	9.97	100.3%	
150%	32998 67	13.05	12.93	100.9%	

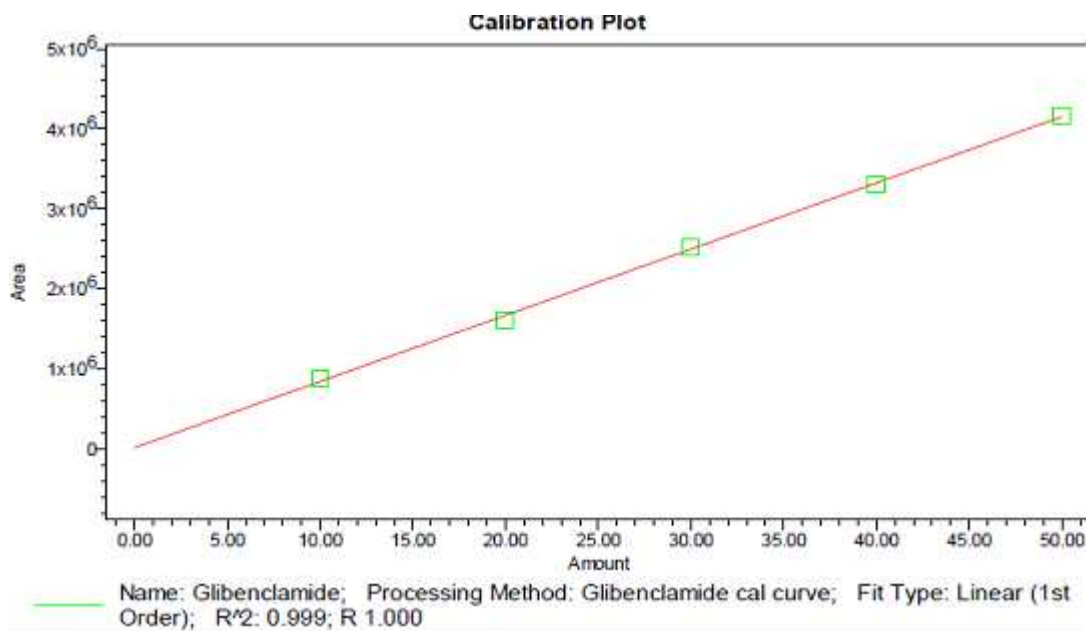
Linearity:

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for Glibenclamide found to be as 10-50mcg/ml. The regression equation for $y = 82682x + 14082$ with correlation coefficient (R^2) 0.999 (table no.4, Figure no.1).

Table No-4: Summary of Linearity.

S.No	Linearity Level	Concentration	Area
1	I	10µg/ml	877298
2	II	20µg/ml	1606180
3	III	30µg/ml	2519518
4	IV	40µg/ml	3310612
5	V	50 µg/ml	4159223
Correlation Coefficient			1.000

Figure No-1: Linearity Graph.



LOD and LOQ:

The LOD concentration obtained is 0.0000264mg/ml (or) 0.8% with respect to working concentration of 0.03mg/ml. The LOQ concentration obtained is 0.00007mg/ml (or) 2.5% with respect of working concentration of 0.08mg/ml (table no.5 and 6).

Table No-5: Summary of LOD.

Component	Working conc. (mg/ml)	LOD Conc. (mg/ml)	Signal To Noise Ratio
Glibenclamide	0.03	0.000024	2.8

Table No-6: Summary of LOQ.

Component	Working conc. (mg/ml)	LOD Conc. (mg/ml)	Signal To Noise Ratio
Glibenclamide	0.03	0.00007	10.32

Robustness:

The Robustness of method as carried out by changing the Chromatographic conditions such as change in organic phase composition in mobile phase and flow rate variations. With the change of composition of mobile phase 10% less, actual, 10% more with organic phase and change in flow rate of 0.6, 0.8 and 1.0 ml their USP Tailing factor, USP plate count was calculated (table no.7, and8).

Table No-7: Summary of Robustness.

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2653	1.2
2	*Actual	2545	1.25
3	10% more	2465	1.3

Table No-8: Summary of Robustness.

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	2708	1.2
2	0.8	2545	1.3
3	1.0	2354	1.35

Conclusion:

The proposed method is simple, specific, accurate and precise and hence can be used in routine for estimation of Glibenclamide in tablet dosage. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The percentageRSD for all parameters was found to be less than two, which indicates the validity of the method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of Glibenclamide in tablet dosage form.

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