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A RP-HPLC Method Development and Validation for the Estimation of Glibenclamide in Bulk and Pharmaceutical Dosage forms

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Glibenclamide in pharmaceutical dosage form. Isocratic elution at a flow rate of 1.0 mL min $^{-1}$ was employed on Inertsil-Extend C_{18} column at ambient temperature. The mobile phase consisted of methanol: phosphate buffer 45:55 (v/v) and the detection wavelength were at 228 nm. Linearity was observed in concentration range of 10-150 µg/mL. The retention time for Glibenclamide was 10.43 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Glibenclamide in pharmaceutical dosage forms.

Key words: *Dosage forms, Estimation, Method development, Glibenclamide, RP-HPLC, Validation.*

INTRODUCTION

Glibenclamide (**Fig. 1**) also known as glyburide is a potent, second generation oral sulfonylurea antidiabetic agent widely used to lower blood glucose levels in patients with type II non-insulin-dependent diabetes mellitus and as well as in gestational diabetes. It acts mainly by inhibiting the sulfonylurea receptor and stimulating endogenous insulin release from beta cells of pancreas [1].Chemically it is described as 5-chloro-*N*-(4-[*N*-(cyclohexylcarbamoyl) sulfamoyl] phenethyl) -2-methoxybenzamide.

Literature survey reveals that few spectrophotometric methods [2-4], HPLC methods [5-10], HPTLC method [11] has been reported for the estimation of Glibenclamide. The aim of the present study is to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of Glibenclamide in pharmaceutical dosage form as per ICH guidelines.

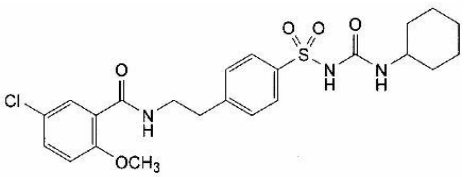

Fig. 1: Chemical structure of Glibenclamide

MATERIALS AND METHOD

Instrumental and analytical conditions:

The HPLC analysis was carried out on YOUNGLIN ACME 9000(Korea) HPLC system equipped with Autochrome 3000 integrater and UV detector. The column used is Inertsil-Extend C_{18} (250 × 4.6 mm, packed with 5 µm) and detection was performed at 228 nm. The injection volume of sample was 15 µL and the run time was 15 minutes. An isocratic mobile phase containing methanol and 0.02 M phosphate buffer at 45: 55 (v/v) at the pH 3.2 was carried with the flow rate at 1.0mL min^{-1}. The mobile phase was filtered through 0.45µm membrane filter and degassed before use.

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Reagents and chemicals:

Glibenclamide working standard was kindly gifted by Dr Reddy's Laboratories, Hyderabad. Tablets were purchased from local pharmacy manufactured by Cadila Pharmaceutical Ltd (Afdiex). Ultra pure water was obtained from a millipore system. HPLC grade methanol was obtained from Merck (India) limited. All other chemicals used were AR grade.

Preparation of mobile phase:

Dissolved 2.72 g of Potassium Di hydrogen orthophosphate in 1000 mL of water and mixed, pH adjusted to 3.2 using ortho phosphoric acid, sonicated to degas the buffer. Transferred 450 volumes of methanol and 550 volumes of buffer into a 1000 volumes mobile phase bottle and mixed. Then sonicated up to 15 minutes for degas the mobile phase and filtered through 0.45 µm filter under vacuum. The same mobile phase was used as diluent.

Preparation of Standard Solution:

Accurately weighed about 10 mg of Glibenclamide and transferred into a 10mL volumetric flask and 5 mL of methanol was added and sonicate to dissolve it completely and then volume was adjusted with methanol to get stock solution of 1000 µg/mL. Then 1 mL of stock solution is transferred into 10 ml volumetric flask and make up to volume with mobile phase and filter through 0.45 µm filters, which gives a solution of strength 100 µg/mL.

Preparation of sample solution:

Weigh 20 Glibenclamide tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 50 mg of Glibenclamide into a 50 ml volumetric flask. Add about 25ml of methanol, sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm filter. Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter.

Method Validation

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, limit of detection, limit of quantification, robustness and system suitability.

Linearity:

From the standard stock solution, the various dilutions of Glibenclamide in the concentration of 10, 25, 50, 75, 100 and 150µg/mL were prepared. The solutions were injected using 15 µL injection volumes in to the chromatographic system at the flow rate of 1.0 ml/min

and the effluents were monitored at 228 nm, chromatograms were recorded. Calibration curve of Glibenclamide was obtained by plotting the peak area ratio versus the applied concentrations of Glibenclamide, given in **Table. 1**. The linear correlation coefficient was found to be 0.999, shown in **Fig. 2**.

Table No. 1: Linearity of Glibenclamide

Concentration (µg/mL)	Average area
10	315494
25	937790
50	1812480
75	2750059
100	3540118
150	5273843

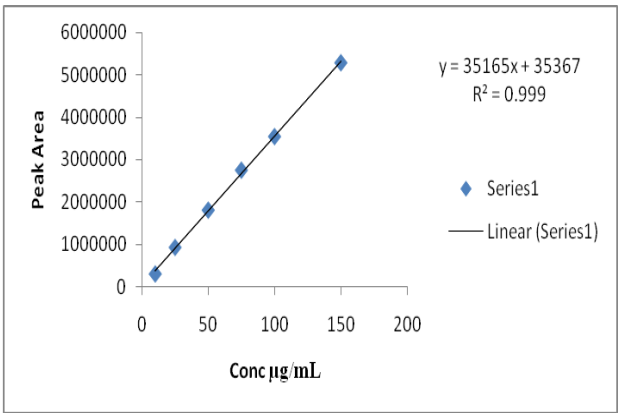

Figure 2: Linearity curve of Glibenclamide

Precision:

Repeatability of the method was checked by injecting replicate injections of 100 µg/mL of the solution for six times on the same day as intraday precision study of Glibenclamide and the % RSD was found to be 0.09, given in **Table. 2**.

Table No. 2: Precision of Glibenclamide

Injections	Area
1	3548253
2	3541643
3	3547118
4	3546892
5	3544818
6	3540118
Mean	3544807
SD	3272.938
% RSD	0.09233

Accuracy:

Glibenclamide reference standards were accurately weighed and added to a mixture of the tablets excipients, at three different concentration levels (50%, 100% and 150%). At each level, samples were prepared in triplicate and the recovery percentage was determined and presented in **Table 3**.

Table No. 3: Accuracy of Glibenclamide

%Conc	Amount added (mg)	Amount found (mg)	% Recovery	Mean Recovery
50%	5.0	4.96	99.2 %	
100%	10.0	9.96	99.6%	99.5 %
150%	15.0	14.95	99.7 %	

Specificity:

Spectral purities of Glibenclamide chromatographic peaks were evaluated for the interference of the tablet excipients as per the methodology. In the work, a solution containing a mixture of the tablet excipients was prepared using the sample preparation procedure to evaluate possible interfering peaks and no interference peaks were observed.

Robustness:

To determine the robustness of the method, two parameters (flow rate, composition of mobile phase) from the optimized chromatographic conditions were varied. Statistical analysis showed no significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of parameters were introduced. Thus the method showed to be robust which is shown in **Table. 4**.

Table No. 4: Robustness of Glibenclamide

Parameters	Adjusted to	Average Area	R_t	SD	% RSD
Flow rate as per method 1.0mL/min	0.9 mL/min	3716254	10.43	7918.5	0.21
	As it is	3538923	10.42	2507	0.07
	1.1ml/min	3569358	10.38	9899.6	0.27
Mobile phase composition Methanol:Buffer (45:55)	Methanol:Buffer (40:60)	3615523	10.41	4972.4	0.13
	As it is	3588956	10.42	3587.8	0.10
	Methanol:Buffer (50:50)	3642412	10.41	8995.8	0.24

Ruggedness:

Inter day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation and statistical analysis showed no significant difference between results obtained employing different analyst.

Detection and Quantitation limits:

According to the determined signal-to-noise ratio, Glibenclamide presented limits of detection of 0.09 µg/mL and limits of quantitation of 0.3 µg/mL, where the compounds proportion found in the sample solutions injected on to the chromatograph. However, the objective of the method is the quantitation of Glibenclamide so that the values obtained should be considered as the limit of method sensitivity.

System Suitability:

System suitability tests were carried out on freshly prepared standard stock solutions of Glibenclamide and it was calculated by determining the standard deviation by injecting standards in six replicates at 6 minutes interval and the values were recorded and the system suitability parameters are shown in **Table. 5**.

Table No. 5: System Suitability of Glibenclamide

Concentration	Injection	Area	R_t
100ppm	Inj-1	3551839	10.437
	Inj-2	3546824	10.433
	Inj-3	3550638	10.434
	Inj-4	3541788	10.436
	Inj-5	3543158	10.435
	Inj-6	3547218	10.436
Statistical Analysis	Mean	3546911	10.43517
	SD	3965.04	0.001472
	% RSD	0.11	0.014
	Tailing Factor	1.009	
	Plate Count	6583.9	

Assay of Glibenclamide tablet:

Three different batches of Afdiex were analyzed using the validated method. For the analysis, six replicates of each batch were

assayed. Twenty tablets were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 50mg of Glibenclamide was transferred to a 50 ml volumetric flask followed by the addition of 25 ml of methanol. The solution was sonicated for 3 minutes and volume adjusted with the mobile phase then filtered through 0.45 µm membrane filter. Further dilutions were made to get the final concentration equivalent to 100 µg/mL of Glibenclamide. The mean peak area of the drug was calculated and the drug content in the tablets was quantified and the results were presented in **Table. 6**.

All the analyzed batches presented Glibenclamide were very close to the labeled amount. The Glibenclamide content in the tablets samples varied from 99.2 to 99.6%.

Table No. 6: Contents of Glibenclamide in tablets (n=6)

Sample tablet	Batch	Labeled Amount (mg)	Amount found(mg)±SD	%Amount found
	1	5	4.98±0.11	99.6
Afdiex	2	5	4.96±0.15	99.2
(5mg)	3	5	4.98±0.08	99.6

S.D=Standard Deviation

RESULTS AND DISCUSSION

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug Glibenclamide was preferably analyzed by reverse phase chromatography and accordingly C_{18} column was selected. The elution of the compound from the column was influenced by polar mobile phase. The ratio of the methanol to phosphate buffer was optimized to give symmetric peak with short run time. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase of methanol: phosphate buffer at the ratio of 45:55 (v/v).The retention time of Glibenclamide was found to be 10.43 min, which indicates a good base line. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability parameters are given in Table 5. Developed chromatographic method was applied for the determination of Glibenclamide in tablet formulation, given in **Table. 7**. A typical chromatogram showing the separation of Glibenclamide is shown in **Fig. 3**.

Table No. 7: Developed Chromatographic Conditions

Parameters	Method
Stationary phase (column)	Inertsil-Extend C_{18} (250 × 4.6 mm, packed with 5 µm)
Mobile Phase	45:55 (Methanol : Phosphate Buffer)
pH	3.2 ± 0.02
Flow rate (ml/min)	1.0
Run time (minutes)	15.0
Column temperature (°C)	Ambient
Volume of injection loop (µl)	15
Detection wavelength (nm)	228
Drugs Rt (min)	10.43

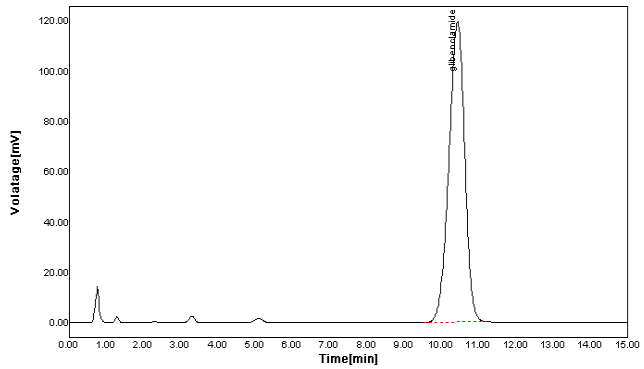

Fig. 3: Standard Chromatogram of Glibenclamide.

CONCLUSIONS

A validated RP-HPLC method has been developed for the determination of Glibenclamide in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Therefore, it is suitable for the routine analysis of Glibenclamide in pharmaceutical dosage form.

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