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RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF GLICLAZIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A Simple, accurate and rapid RP-HPLC method has been developed for the estimation of Gliclazide (GLC) in bulk and pharmaceutical dosage forms using an XDB C₁₈, 150 x 4.6 mm i.d, 5 µm particle size in isocratic mode; with mobile phase comprising of buffer (0.02M potassium dihydrogen phosphate) and acetonitrile in the ratio 55:45 (v/v). The flow rate was 1 ml/min and detection was carried out by photodiode array detector at 228 nm. The retention time for GLIC was found to be 5.078 min. The proposed method has permitted the quantification of GLIC over linearity in the range of 25 – 150 µg/ml and its percentage recovery was found to be 99.21 – 100.419 %. The % RSD of intraday and inter day precision were found 0.4433% and 0.1%. This RP – HPLC method is simple, single and reproducible, with high resolution and has been successfully applied for the determination of GLC.

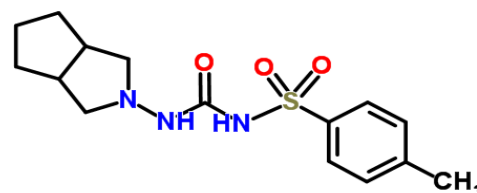
Keywords: Gliclazide, RP-HPLC, Validation and method validation.

INTRODUCTION

GLC is a sulfonylurea antidiabetic. It is given by mouth in the treatment of type-2 diabetes mellitus. Chemically, GLC is 1-(Hexahydrocyclopental[c]pyrrol-2(1H)-yl)-3-[(4-methyl-phenyl) sulfonyl] urea with empirical formula of C₁₅H₂₁N₃O₃S and molecular weight 323.4 [1,3]. The chemical structure of GLC was shown in figure.1. GLC is readily absorbed from the gastrointestinal tract. It is extensively bound to plasma proteins. The half life is about 10 to 12 hours. GLIC is extensively metabolized in the liver to metabolites that have no significant hypoglycaemic activity. Metabolites and a small amount of unchanged drug are excreted in the urine [2,4,5]. Non aqueous method have been reported for analysis of gliclazide [1] in BP. A survey of literature has revealed few UV spectrophotometric methods for individual and simultaneous estimation of Gliclazide in Pharmaceutical formulation [6,7]. Several HPLC

procedures have been reported for the determination of Gliclazide in bulk, pharmaceutical dosage forms and biological fluids [8-10]. Because of the relatively high polarity (pKa = 5.8, log P =2.1) [11] most of the methods published in literature used in liquid – liquid extraction but solid phase extraction [10] and mild protein precipitation [12, 13] of the plasma sample were also reported. We here in report a simple, rapid and reliable RP-HPLC for the estimation of GLC in bulk and pharmaceutical dosage forms.

Fig 1. Structure of Gliclazide



Experimental Reagents and Materials

Pure standard of GLIC (99.7%) was obtained as gift sample from Ranboxy Pharma Ltd, New delhi. HPLC grade acetonitrile (Rankem, avantor chemicals, gujarat), HPLC grade water, potassium dihydrogen phosphate (merck specialities pvt ltd, mumbai), ortho phosphoric acid (merck specialities pvt ltd, mumbai), RECLIDE tablets (Dr.reddy's Pharma Ltd), high precision weighing balance (wensar instruments, hyderabad), micro pipette (in labs, 10-100 μ l) were employed in the study. All the glassware employed in the work cleaned with hot water followed acetic anhydride then acetone and dried in hot air oven whenever required. Working environment was maintained in between 18-22°C. However, the chemical structure and purity of the sample obtained were confirmed by melting point, TLC Studies.

HPLC Apparatus and Chromatographic Conditions

The analysis was performed on Waters 2695 HPLC system with Waters 2996 Photodiode Array detector. Data acquisition was performed by using Empower 2 software. XDB, C18 column (150 x 4.6mm, 5 μ) was used as stationary phase. Injections were performed by the manual injector with 10 μ l. Different mobile phases were tested in order of their polarity to find out the best conditions for the separation of gliclazide. The selected mobile phase Acetonitrile and Potassium Dihydrogen Phosphate buffer of 0.02M (pH 3.1) in the ratio of 45:55 v/v gave acceptable retention time (RT). The flow rate was maintained at 1.0 mL min⁻¹, with a run time of 10 min. the mobile phase was filtered by using 0.45 μ filter and it was degassed by sonication prior to use. All determinations were made at ambient temperature. The eluent was detected at 228nm.

PROCEDURE RECOMMENDED

Preparation of Mobile

Potassium Dihydrogen Phosphate Buffer (0.02M KH₂PO₄) and acetonitrile taken in the ratio 55:45 (v/v) were employed as a mobile phase.

Preparation of Stock Solution

A stock solution was prepared by accurately weighed and transferred 15mg of GLIC working standards into a 10ml clean dry volumetric flask, add 7ml of diluent (Water and acetonitrile 80:20), sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution, 1ml was pipette out into a 10ml volumetric flask and then make up to the final volume with diluent.

Construction of Linearity

The concentrations of analyte were prepared from the stock solution by taking suitable volume (0.25 – 1.5 ml) and diluted up to 10 ml to get the desired concentrations for linearity in the range of 25 – 150 μ g/ml.

the prepared solutions were filtered through 0.45 μ m membrane filter and each of the dilutions was injected three times into the column. The calibration curve for GLC was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis). It was found to be linear in the concentration range 25-150 μ g/ml with good correlation in between concentration and mean peak area.

Estimation of GLC

20 tablets were weighed and the contents were removed to obtain the average weight powder. A sample of the powder claimed to contain 50mg of active ingredient, was mixed with 70ml of diluent. The mixture was allowed to stand with intermittent sonication to ensure complete solubility of drug. Further the volume made up with diluent and the resulting solution was passed through 0.45 μ m membrane filtered. From the filtered stock solution of 1mg/ml an aliquot of this solution (0.5ml) was transferred to a volumetric flask and made up to a sufficient volume with diluent to get desired concentration of 25 μ g/ml. the prepared dilution was injected three times into the column to obtain chromatogram. From that peak area, the drug content in the tablet was quantified.

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. Validated the developed method as per ICH & FDA [14-18] guidelines with parameter like Specificity, precision, accuracy, linearity and range, ruggedness and robustness, etc.,

RESULTS

The present RP-HPLC method for the quantification of GLC in bulk and pharmaceutical dosage forms, revealed as simple, rapid, accurate and precise method with significant shorter retention time of 5.078.min. The linearity for the detection of GLC was 25-150 μ g/ml with ($R^2 = 0.999$; $y = 15688x + 369.1$) the coefficients of variation based on mean peak area for three replicate injections were found to be 0.999. Results were shown in table-1 and statistical data of calibration curves were shown in table-2. The intraday and inter day variations of the method were determined using five replicate injections analyzed on the same day and next day over a period of 24 hours. The result revealed the precision with %RSD of 0.4433 and 0.1, respectively for intraday and inter day. Results were shown in table-3. To ensure the reliability and accuracy of the method, the recovery studies were carried out by adding a known quantity of drug with pre-analyzed sample and contents were reanalyzed by the proposed method. Accuracy was

evaluated by injecting the solution about three times, at three different concentrations equivalent to 50%, 100% and 150% of the active ingredients, by adding a known amount of GLIC standard to a sample of known concentration and calculating the recovery of GLC with RSD (%) and recovery for each concentration. The mean % recoveries were in between 99.12 – 101.41% and were given in table- 4. The assay for the marketed tablets of RECLIDE tablets (Dr.Reddy’s Pharma Ltd) was established with present chromatographic condition developed and it was found to be more accurate and reliable. The average drug content was found to be 100.13 of the labeled claim and no interfering peaks were found in chromatogram, indicating that the estimation of drug free from inference of excipients. The results were shown in table – 5. To know reproducibility of the method system suitability test was employed to establish the parameters such as tailing factors, theoretical plates, limit of detection and limit of quantification and values were shown in table-6. Ruggedness of the method (intermediate precision) was estimated by preparing six Dilutions of the GLIC as per the proposed method and each dilution injected into

column. The results were shown in table -7. Robustness of the proposed method was estimated by changing mobile phase composition from buffer: acetonitrile (55:45) v/v to buffer: acetonitrile 60:40 (v/v), changing the flow rate from 1ml to 1.2 ml/min, changing the temperature ($\pm 5^{\circ}\text{C}$) and system suitability parameters were found to be within acceptable limits [19]. Results were shown in table-8 and indicating that the test method was robust for all variable conditions. Hence the method was sufficiently robust for normally expected variations in chromatographic conditions. The ruggedness and robustness for the method was performed as per ICH guidelines. Limit of detection (LOD) and quantification (LOQ), the limits of detection and quantification were calculated by the method based on the standard deviation (σ) and the slope (S) of the calibration plot, using the formulae $\text{LOD} = 3.3 \sigma/s$ $\text{LOQ} = 10 \sigma/s$. The specificity test of the proposed method demonstrated that the excipients from tablets do not interfere in the drug peak. Furthermore, well shaped peaks indicate the specificity of the method. The typical chromatograms of GLC standard and tablet dosage form were shown in figure 2,3.

Table 1. Concentration Vs Mean Peak Area

Concentration ($\mu\text{g/ml}$)	Mean peak area
37.5	599300
75	1173373
112.5	1729215
150	2387423
187.5	2930067
225	3532219
Regression Equation	$y = 15688x - 369.1$
Correlation coefficient (r^2)	0.999

Table 2. Statistical Data of Calibration Curves of GLC

Parameters	GLC
Linearity	37.5 – 225 $\mu\text{g/ml}$
Regression Equation	$15688x - 369$
Average of Slope	15688
Average of intercept	369.1
Standard deviation of intercept	1563.46
Correlation coefficient (r^2)	0.999

Table 3. Precision of method

Parameters (n=6)	Intraday	Interday
Mean Peak Area	2606073	2391526
Standard deviation	11553.01	2595.3
%RSD	0.44	0.1

Table 4. Recovery Study of Method

Standard of Drug (μg)	% of drug Added	Amount Present(μg)	Mean amount found (n=3)	Mean % recovery
150	50	75	75.28 ± 0.08	100.39
150	100	150	149.96 ± 0.09	99.97
150	150	225	225.06 ± 0.08	100.02

Average mean % recovery = 100.13, Standard deviation = 0.647, %RSD = 0.646

Table 5. Estimation of amount of GLC

Brand name of Tablet	Label claim (mg)	Amount of drug estimated (%)	Mean amount	%RSD
Reclide	50	99.59	99.59± 0.441	0.44

Table 6. System suitability Parameters

Parameters	GLC
Retention time (min)	5.078
Theoretical plates	6484
Tailing factor	1.02
Linearity Range (µg/ml)	37.5 - 225
Limit of Detection (LOD) (µg/ml)	0.328
Limit of Quantification (LOQ) (µg/ml)	0.996
Relative standard deviation (RSD)	0.999

Table 7. Ruggedness of method

S.No	Labeled Claim (mg)	Amount estimated (%)	Mean ± S.D	%RSD
Analyst - 1	50	100.13	100.13 ± 0.64	0.6
Analyst - 2	50	99.59	99.59 ± 0.44	0.4

Table 8. Robustness of method

Parameter	Variation	Theoretical Plates	Tailing factor	%RSD
Standard	-	6411	1.02	0.44
Flow rate	0.8 ml	7123	1.03	0.1
	1.2 ml	6796	1.03	1.7
Mobile phase	50:50	6678	1.03	0.1
	60:40	6672	1.03	0.1
Temperature	-5°C	6812	1.03	0.7
	+5°C	6913	1.03	0.0

Fig 2. A typical chromatogram of Gliclazide

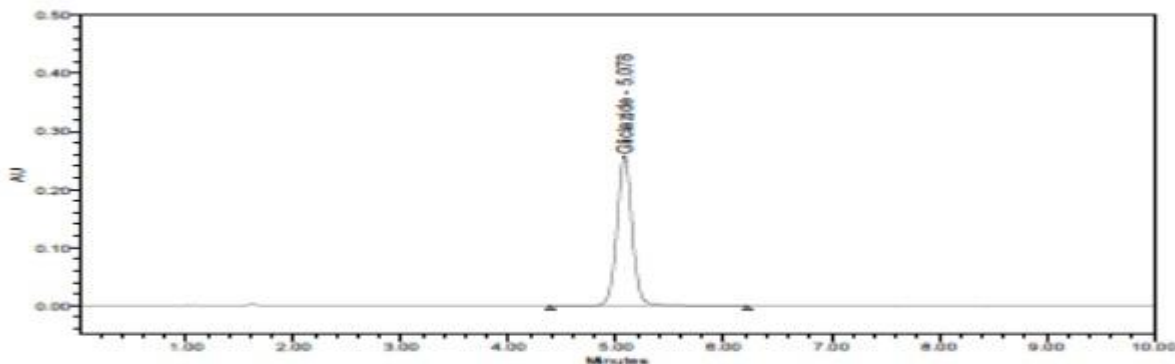
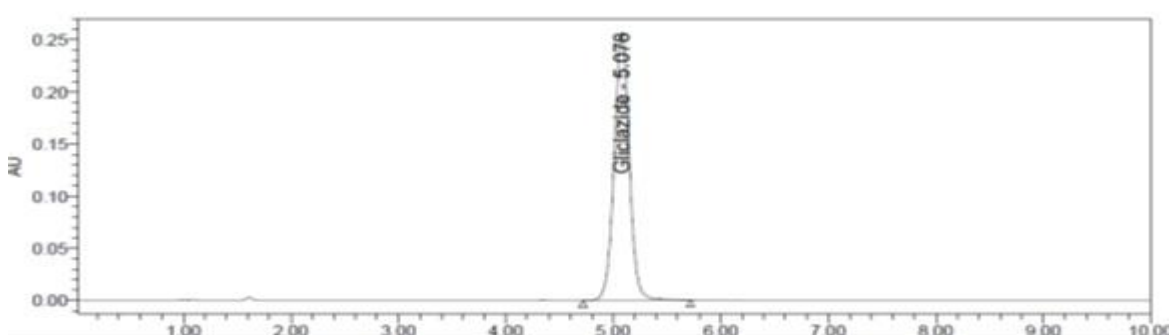


Fig 3. Chromatogram showing the assay of Gliclazide marketed dosage form (RECLIDE tablets).



DISCUSSION

The development of HPLC methods for the determination of drugs has received considerable attention in recent years because of their importance in the quality control of drugs and drug products. The goal of this study was to develop and validate a RP-HPLC method for the estimation of GLC in bulk and pharmaceutical commercial preparations. The main objective of method development was to determine the drug content present in the formulation and its % purity. The chromatographic conditions like mobile phase composition, flow rate was optimized and the method was developed, validated successfully. The selected mobile phase system gave a single sharp peak without interfering peaks. Initial development of the method various mobile phases were tried to get sharp peak, finally buffer: acetonitrile in the ratio of 55:45 (v/v) was selected which gave a single sharp peak with retention of 5.078 min and tailing factor 1.02. Commercial marketed formulation of GLC was analyzed for its contents and % of content was calculated. The proposed method was found to be simple, rapid, economic and accurate and the method was applicable to routine laboratory analysis. The method was validated statistically for various parameters like standard deviation, % relative standard deviation, slope and intercept. The proposed

method was following linearity in the concentration range of 25-150 µg/ml and obeys the beers lamberts law and above 25-150 µg/ml the linear plot showing deviation from beers law. Every concentration was injected into chromatographic system about three times and peak areas were noted. Greater reproducibility was obtained for calibration plots and it was determined by calculating the slope, intercept and %RSD for each standard plot. The method was found to be robust as there was no significant change in the peak area and retention time. The system suitability tests were performed to assess the quality performance of the method. The method was found to be more specific, robust and rugged and most suitable for routine analysis.

CONCLUSION

The proposed single and high resolution RP-HPLC method was validated with linearity, precision, accuracy and specificity. The complete results proved that the developed method to be convenient and effective for the determination of all active ingredients during the analysis of the bulk as well as pharmaceutical dosage forms. Moreover, this single, short analysis time and high resolution RP-HPLC method for the estimation of GLC leads to cost effective chromatographic method.

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