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Original Research Article

METHOD DEVELOPMENT AND VALIDATION OF GLIBENCLAMIDE IN TABLET DOSAGE FORM BY USING RP-HPLC

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Abstract:
A simple, precise, reliable, rapid and reproducible reversed phase–high-performance liquid chromatography method was developed and validated for the estimation of glibenclamide. Chromatographic separation was achieved isocratically with Grace Vydac C_{18} column (250 × 4.6 mm, 5µ) and Acetonitrile:20 mM ammonium acetate buffer, pH 4.5 (50:50) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 300 nm. Parameters such as linearity, precision, accuracy, robustness recovery are studied as per ICH guidelines. The retention time for Glibenclamide was found to be 15 min. Linearity for glibenclamidewas in the range of10–70 µg/ml and mean recoveries obtained for glibenclamide was99.22±1.07% and relative standard deviation (RSD) was0.90. The correlation coefficients for all components were close to 1. Developed method was found to be accurate, precise, selective and rapid for estimation of glibenclamide.

Keywords: Glibenclamide, RP-HPLC, validation

Introduction

Glibenclamide, chemically1-{4-[2-(5-chloro-2-methoxybenzmido)ethyl}benzenesulphonyl}-3-cyclohexylurea belongs to the class of sulfonylureas has hypoglycemic activity and is used for the treatment ofnon insulin dependent diabetes mellitus[1].Glibenclamide is a white crystalline powderwith a pKa of 5.3 and a partition coefficient of 4.8 determined in octanol-water.The half-life of the drug is 1.4-1.8 h (0.7-3 h). The usual initial adult dosage is 2.5-5 mg daily. The adult maintenance dose for type II diabetes ranges from 1.25-20 mg daily. The maximum recommended dosage is 20 mg daily[2,3]. The principal action of glibenclamide is on beta cells, stimulating insulin secretion from pancreatic cells and thus reducing plasma glucose. After prolonged administration, the hypoglycemic effects of the drug appear to be related to extra-pancreatic effects including reduction of basal hepatic glucose production and increased peripheral sensitivity to insulin, the latter may result either from an increase in

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the number of insulin receptors or from changes in events subsequent to insulin binding[4,5,6].The present study was aimed for the development and validation of an analytical method for estimation of glibenclamidein tablet dosage form by RP-HPCL.

Materials and Methods

Instrumentation

An HPLC equipped with UV detector was used for the present research work. The separation was achieved using Grace Vydac C_{18} column (250 x 4.6 mm, 5µ).

Chemicals and reagents

Glibenclamide was obtained as a gift sample from US Vitamins, Mumbai. All chemicals and reagents used were of analytical grade. HPLC grade water was used to prepare all solutions.

Method development

Stock and standard solution: Stock solutions of glibenclamide working standard were prepared by dissolving 10 mg of the drug in 10 ml of methanol, so that the final concentration is 1 mg/ ml. From the stock solution 5, 10, 15, 20, 25 µg mL^{-1} dilutions were prepared by using methanol as diluents.

Sample preparation: Diluted concentration of 10 µg/ml glibenclamide was prepared from the primary stock solution using methanol as a diluent. Acetonitrile was selected as organic solvent to elute glibenclamide from the stationary phase because of its favorable UV transmittance, low viscosity and low backpressure.

Chromatographic condition

The isocratic mobile phase consisted of Acetonitrile:20 mM ammonium acetate buffer, pH 4.5 in the ratio of (50:50 v/v), flowing through the column at a constant flow rate of 1.0 ml/ min. Grace Vydac C_{18} column (250 × 4.6 mm, 5µ) was used as the stationary phase. 300 nm was selected as the detection wavelength for detector.

Effect of pH, composition, ionic strength, flow rate of mobile phase

The mobile phases with different pH conditions (pH 3.0 to pH 7.0) were used and retention time was noted. Various compositions of mobile phase were studied in 50:50, 60:40 and 70:30 (% v/v) to optimize the composition of the mobile phase. Different strength of ammonium acetate buffer (pH 4.5) such as 10, 20, 30 and 40 mM and varied mobile phase flow rate such as 0.9, 1.0 and 1.1 ml/min were used to optimize the chromatographic conditions.

Method validation

Linearity

The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte. 0.5, 1, 2, 5, 10, 25, and 50 µg/ml of glibenclamide was prepared in mobile phase and 20 µl of the solution was injected and chromatograms were recorded and regression equation was calculated.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted as true value or reference value. A known amount of standard drug was spiked (100, 120, 150%) in triplicate to the preanalyzed samples and the recovery of the drug was calculated at 10, 12, 15 µg/ml.

Robustness

Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. One consequence of the evaluation of robustness should be that a series of system suitability parameters is established to ensure that the analytical procedure is maintained whenever used.

Precision

Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. It is also termed as intra-assay precision. 10 µg/ml of glibenclamide was analyzed at different time intervals. The percentage relative standard deviation was then calculated.

Intermediate precision

Precision is the measure of the degree of repeatability of an analytical method under

normal operation and is generally expressed as the percent relative standard deviation for a statistically significant number of samples. It was carried out in between days and by different analysts.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD is the ability of analytical method able to detect the lowest concentration of the analyte. LOQ is the lowest concentration of the analyte which can be quantitatively analyzed with acceptable precision and accuracy. It was calculated based on the slope and blank response from the calibration curve as per ICH guidelines. LOD and LOQ were calculated based on the standard deviation of the response and slope.

LOD= 3.3 × SD/S

LOQ= 10 × SD/S

SD: Standard deviation of blank response;

S: Slope of the calibration curve

Results and Discussion

Effect of pH, composition, ionic strength, flow rate of mobile phase

The retention time of glibenclamide was decreased with increase in pH of the mobile phase. This might be due to the unionization of the drug. Hence, Ammonium acetate buffer, pH of 4.5 was selected since elution was within 15 min with adequate system suitability. With a different mobile phase ratio the retention time was found to be 8.4, 7.5 and 4.2 min respectively. On increasing the % of acetonitrile there was a decrease in the retention time which might be due to the higher elution strength of acetonitrile.The retention time of glibenclamide did not change much with the change in buffer strength. So, 20 mM strength of the Ammonium acetate buffer (pH 4.5) was selected. Symmetrical peaks were obtained with the different flow rates. For the present study, 1 ml/min was selected.

Based on the above optimization parameters, the following chromatographic condition was selected for the estimation of glibeclamide by HPLC method.

Stationary phase	: Grace Vydac C_{18} column (250 × 4.6 mm, 5µ)
Mobile phase	: Acetonitrile:20 mM ammonium acetate buffer, pH 4.5 (60:40)
Solvent ratio	: Isocratic run for 30 min
Detection wavelength: 300 nm	
Flow rate	: 1 ml/min
Injection volume	: 20 µl
Temperature	:Ambient (around 25 °C) Auto sampler
Temperature	: 4±2 °C

With the above separation condition, the retention time for glibenclamide was found to be 7.5 mins. The typical standard chromatogram of glibenclamide was shown in Fig.1.

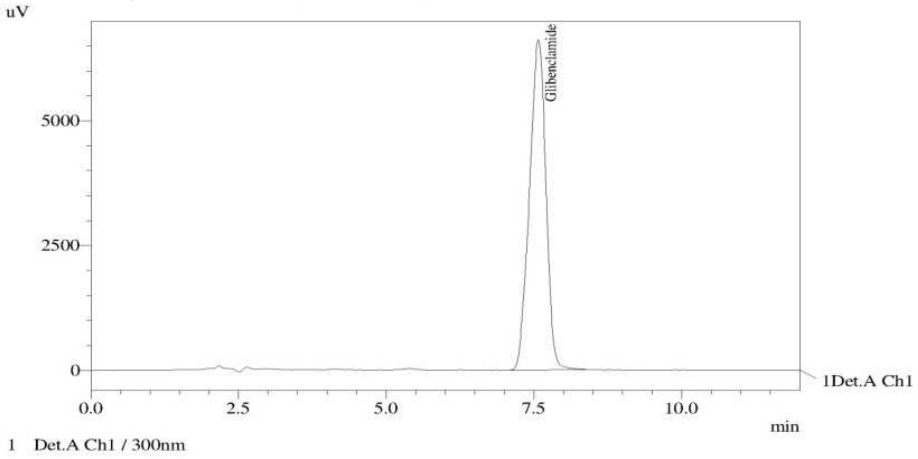

Fig.1. Standard chromatogram of glibenclamide

Linearity

Linearity is generally reported by the coefficient determination (r^2) and the acceptance criteria is (r^2) should be >0.999. The coefficient determination (r^2) was 0.9997 which indicated that the present method is linear (range10–70 µg/ml).(Fig. 2)

Accuracy

Recoveries at three different concentrations (10, 12, 15 µg/ml) were found to be within the range of 98 to 102% as per ICH guidelines. Mean % recovery (Mean±SD) was found to be 99.22±1.07.

Robustness

Overall percentage relative standard deviation was found to be 0.90% and the acceptance limit was <2% which indicated that the method was robust.

Precision

The repeatability and intermediate precision of the proposed method was found to be 0.56% and 0.72% respectively which were within the acceptance criteria indicating that the method was precise and reproducible one.

LOD and LOQ: LOD and LOQ were found to be 0.1 and 0.5 µg/mlwhich suggests that the developed method is sensitive for the quantification of glibenclamide. Summary of the analytical method validation parameters is reported in Table1 as given below.

Table 1. Data of analytical method validation of glibenclamide by HPLC

Validation Parameters	Validation results	Acceptance criteria
Linearity(r^2) (10-70 µg/ml)	0.9997	> 0.999
Accuracy (% Mean±SD)	99.22±1.07	98–102
Robustness (% RSD)	0.90	<2
Repeatability precision (% RSD)	0.56	<1
Intermediate precision(% RSD)	0.72	<2
LOD (µg/ml)	0.1	S/N ratio should be 3:1
LOQ (µg/ml)	0.5	S/N ratio should be 10:1

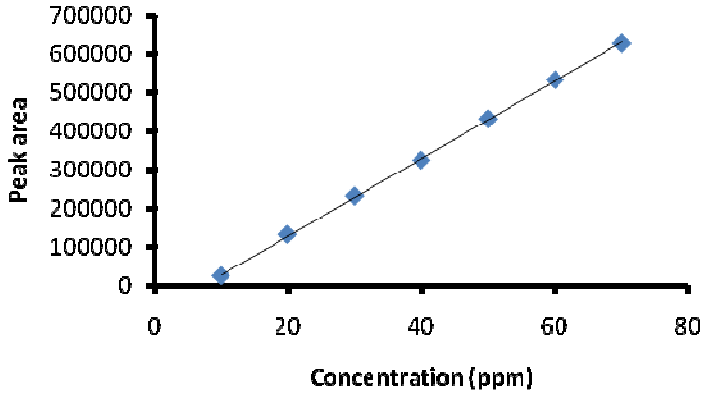

Fig. 2. Standard plot of glibenclamide

Conclusion

RP-HPLC method was developed and validated for estimation of glibenclamide. The developed method is suitable for the identification and quantification of glibenclamide. A high percentage of recovery shows that the method can be successfully used on a routine basis. The proposed method is simple, fast, accurate, precise and sensitive and could be applied for

quality and stability monitoring of glibenclamide.

References

1. Hennessey. J. V., Bustamante. M. A. and Teter. M. L. 1994. Bedtime dosing of glyburide and the treatment of type II diabetes mellitus. Amer J Med Sci.308:234-238.
2. McEvoy. G. K. 2004. AHFS Drug Information. Sulfonylureas. Maryland: American Society of Health-System Pharmacists.
3. Rydberg. T., Jonsson. A. and Karissson. M.O.1997. Concentration-effect relations of glibenclamide and its active metabolites in man: Modelling of pharmacokinetics and pharmacodynamics. Br J Clin Pharmacol. 43:373-381.
4. Rang. H. P., Dale. M. M. Ritter. J. M. and More. P.K. 2003. Pharmacology, New York, Churchill Livingstone. 5th ed., pp 380-393.
5. Davis. S. N., Granner. D. K. 1996. Insulin, oral hypoglycemic agents, and the pharmacotherapy of the endocrine pancreas. In: Hardman, J.G., Limbird, L.E. (Ed.), The pharmacological basis of therapeutics, McGraw-Hill Co., New York. 9th ed., pp1487-1517.
6. Jaber. L. A., Antal. E. J. and Welshman. I. R. 1996. Pharmacokinetics and pharmacodynamics of glyburide in young and elderly patients with non-insulin-dependent diabetes mellitus. Ann Pharmacother. 30:472-475.