INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

HPLC METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF GLIBENCLAMIDE IN PHARMACEUTICAL DOSAGE FORMS

Ashwini M *, Sogali B S, Bhattacharyya S
Department of Pharmaceutics, Krupanidhi College of Pharmacy, Bangalore, India
*Corresponding Author Email: ashwinipreetham2@gmail.com

Article Received on: 10/01/19 Approved for publication: 02/03/19

DOI: 10.7897/2230-8407.1004135

ABSTRACT

Glibenclamide is an oral hypoglycemic agent, which increases the insulin secretion by binding to the sulfonylurea receptors on the beta cells or with ATP sensitive potassium channels on the pancreatic beta cells. The present study includes development of HPLC technique for determination and estimation of glibenclamide in pharmaceutical dosage forms using acetonitrile: phosphate buffer (70:30) as mobile Phase. The detection wavelength of glibenclamide was found to be at 235 nm. The developed method was validated for its linearity, precision, accuracy, specificity, robustness and determination of limit of quantification and limit of detection in the mobile phase. The linearity demonstrated a correlation coefficient of 0.999. Thus, this method can be considered to be precise, reliable, rapid, simple, sensitive and cost effective for determination of glibenclamide in pharmaceutical formulations.

Key words: Glibenclamide, Validation, Hypoglycemic agent, ICH guidelines

INTRODUCTION

Glibenclamide 5-chloro-N-[2-(4 -{[(cyclohexyl carbamoyl) amino] sulfonyl} phenyl) ethyl]-2 methoxybenzamide, 4-[2-(5-chloro-2-methoxybenzamido) ethyl] benzene sulphonamide and Methyl N-4-[2-(5-chloro-2- methoxybenzamido) ethyl] benzene sulphonyl carbamate is an antidiabetic medication belonging to the class of sulfonylureas[1,2]. Glibenclamide is also known as glyburide, it binds to the sulfonylurea receptors on the beta cells or with ATP sensitive potassium channels on the pancreatic beta cells thus increasing the insulin secretion [3]. Glibenclamide is a drug of choice in type 2 diabetes along with life style changes. Recent studies have shown that glibenclamide is effective in lowering blood glucose in women with gestational diabetes also [4,5,6].

Numerous methods are reported for the analysis and determination of glibenclamide in pharmaceutical dosage forms[7]. These reported methods are complex and time consuming; hence there was a need for developing and validating a new method for qualitative and quantitative estimation of glibenclamide in various formulations. The current method consists of simple, rapid, accurate, specific and precise RP-HPLC method for the estimation of glibenclamide in pharmaceutical dosage forms

MATERIALS AND METHODS

Glibenclamide was procured as a gift sample from Mankind Pharma. Methanol and water (HPLC-grade) were obtained from Himedia labs, Bangalore. Daonil (Sanofi-aventis) tablet was purchased from the local pharmacy, Bangalore. All other chemicals and reagents used were of analytical grade.

Chromatographic Condition

The instrument used was a Phenomenex (USA), consisting of a model LC AT VP intelligent solvent delivery pump, 7125-Rheodyne injector, a computerized system controller (with the Baseline chrome Tech N2000 software), and a UV SPD10A detector set at 235 nm. Chromatographic separation was performed using a C-8 Phenomenex HPLC column (Dimension 250X4.6 mm, 5µg). The mobile phase consisted of acetonitrile: phosphate buffer (70:30). The apparent pH of the mixed solvent system was adjusted to 3 ± 0.1.

Method Validation

The methods were validated as per the ICH guidelines on analytical process validation. The linearity, accuracy, precision, specificity, and robustness of the method were validated as per ICH guidelines[8].

Linearity

Stock solution of glibenclamide at a concentration of 100 µg/ml was prepared in the mobiles phase. From the stock several dilutions between 20-80 µg/ml were prepared and injected into the column at a rate of 1 ml/min at injection volume of 20 µL. The calibration curve was plotted using area of retention time versus concentrations µg/ml. linear regression analysis was used to assess the linearity using least square regression method [9].

Precision and Accuracy

The assay precision was carried out by interday and intraday study and the process was evaluated for solutions (20, 40, 60 µg/ml) and were analyzed at different time points. The % RSD (Relative standard deviation) was calculated for the methods[10].

Specificity

As per ICH guideline "Specificity" can be defined as the ability of the method to specifically detect the particular API or analyte

in the presence of other components[11]. A marketed formulation of glibenclamide, Daonil from Sanofi Aventis Pharma India was taken, and specificity was determined in triplicate at a concentration of 50 µg/ml and percentage RSD was calculated[8].

Determination of Robustness

Robustness of an analytical method is necessary to determine the consistency of the process with a minute intentional change in the procedure. This reflects the suitability and reliability of the method in regular use.[12, 13]. The robustness of the RP HPLC method was checked by allowing small deliberate change in injection flow rate (±0.2 units) and wavelength (±1 unit) and percentage RSD was found out.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were determined according to ICH guidelines by the below mentioned formulae [8].

$$LOD = 3.3 * SE / A \quad (1)$$

$$LOQ = 10 * SE / A \quad (2)$$

SE=Standard error of Y intercept. A= Slope of the calibration curve

Table 1: Calibration data of glibenclamide in acetonitrile: phosphate buffer (70:30)

Concentration in µg/ml	Retention time R_t (min)	Peak area
20	3.01	80497.27
40	3.3	156779
60	3.4	232363.7
80	3.34	317274.9

Table 2: Results of precision (Intraday)

Type of solution	Concentration (µg/ml)	Peak area	%RSD
Acetonitrile:phosphate buffer pH 3.0	20	430534.2	0.66
	40	849890.4	
	60	1286014	

Table 3: Results of precision (Interday)

Type of solution	Concentration (µg/ml)	Peak area	%RSD
Acetonitrile:phosphate buffer pH 3.0	20	431124.2	0.69
	40	850594.1	
	60	1287546	

Table 4: Results of accuracy

Concentration added	Concentration recovered	%Mean recovery	%RSD
20	20.14	99.2	0.23
20	20.2		
20	20.1		
40	39.8	100.6459	0.13
40	39.70		
40	39.73		
60	60.3	99.77868	0.25
60	60.12		
60	60.09		

Table 5: Specificity study

Preparation	Area	Concentration	Recovery	%RSD	%Accuracy
50 µg/ml	1109476	50.87	101.73	0.94	102.74±0.97
50 µg/ml	1121346	51.41	102.83		
50 µg/ml	1130331	51.83	103.66		

Table 6: Results of robust (change in injection flow rate)

Injection flow rate 1.2 ml/min				
Concentration	Peak Area	Found concentration	% Recovery	% RSD
20	431753	19.82428	99.1214	1.608925
40	1104225	50.70136	101.4027	
60	1295259	59.47284	99.1214	

Table 7: Results of robust (change in wavelength)

Wavelength 234				
Concentration	Peak Area	Found concentration	%Recovery	% RSD
20	450742.7	20.28819	101.4409	
40	869484.7	39.13601	97.84002	
60	1333657	60.02866	100.0478	1.819825

Table 8: Determination of LOD and LOQ

Statistical parameters	Value (µg/ml)
LOD	**1.61**
LOQ	**4.88**

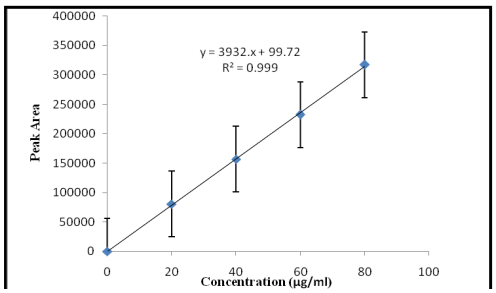

Figure 1: Calibration curve of glibenclamide in acetonitrile: phosphate buffer (70:30)

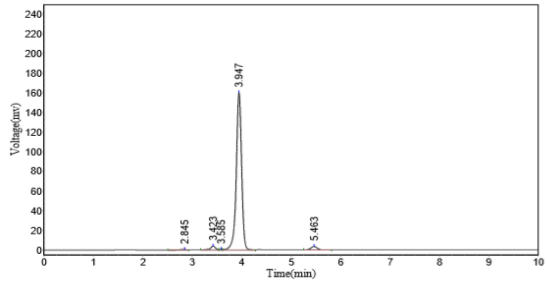

Figure 2: Chromatogram of glibenclamide at 50 µg/ml.

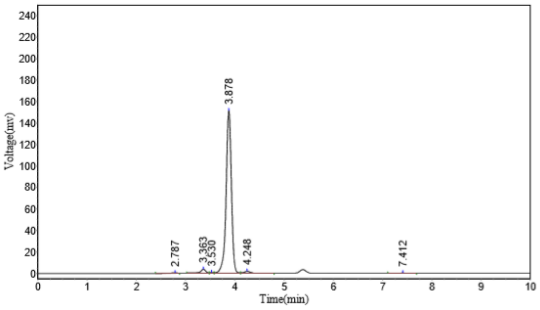

Figure 3: Chromatogram of glibenclamide at 50 µg/ml.

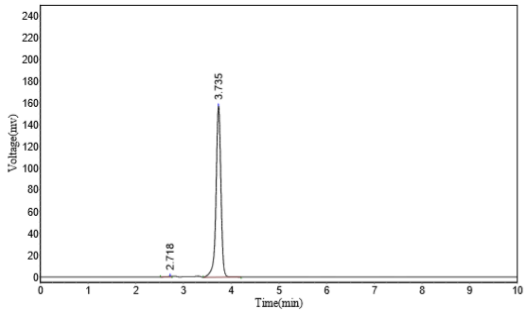

Figure 4: Chromatogram of glibenclamide at 50 µg/ml.

RESULTS AND DISCUSSION

Linearity

The calibration curve for the method obtained was linear over the concentration of glibenclamide 20-80µg/ml. The correlation coefficient obtained was 0.999, thus indicating excellent correlation between peak areas. Calibration data is presented in table 1 and calibration curve shown in figure 1 proves the linearity over the concentration range 20 to 80 µg/ml.

Precision and Accuracy

The interday and intraday variation study of three different dilutions of glibenclamide solutions in acetonitrile: phosphate buffer pH 3.0 (70:30) showed % RSD less than 2% as shown in table 2 and table 3 respectively.

A good accuracy was verified with a mean recovery which was not less than 99 % and not more than 101% for study of the different dilutions of glibenclamide in acetonitrile: phosphate (70:30) buffer pH 3.0 solutions as shown in table 4.

Specificity

Specificity was determined by analyzing marketed sample of glibenclamide (Daonil 5mg tablet). The content was found at an average of 102.74±0.97 % in buffer solution. The % RSD was found to be 0.94 as shown in table 5 and figure 2, 3 and 4. The excipients present in marketed injection did not interfere in the analysis.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain consistent, when subjected to a minute but intentional deviation in the procedure and provides an indication of its reliability during regular usage. Considering a slight change in injection rate and wavelength %RSD was found to be less than 2% as shown in the table 6 and 7.

Limit of Detection and Limit of Quantification

The lowest amount of analyte in a sample that can be detected but not necessarily quantitated LOD (limit of detection) and the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions or quantitation limit LOQ (limit of quantitation) was determined. The LOD and LOQ were found to be **1.61** µg/ml and **4.88** µg/ml as shown in table 8. The significantly low value of LOD and LOQ proved the sensitivity of the process.

CONCLUSION

The developed RP-HPLC method is able to determine glibenclamide in raw materials and in pharmaceutical dosage forms; this can be attributed to the good separation and resolution of the chromatographic peaks with optimum retention time of 3.2 minutes. The results were in good conformity with the affirmed statistical and Pharmacopeial contents. Hence, this method can be considered to be precise, reliable, rapid, simple, sensitive and economical nature.

ACKNOWLEDGEMENT

The authors extend a deep sense of gratitude to management and principal of Krupanidhi College of Pharmacy, Bangalore for providing all necessary support and facility to complete this research work.

REFERENCES

1. Sani AA, Garba Maidugu H, Musa O, Ilyas M. Analysis of some brands of glibenclamide marketed and used in maiduguri metropolis, using ultra violet spectrophotometry and high performance liquid chromatographic (HPLC) methods. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2015; 6(6): 330-338.
2. Ryu RJ, Hays KE, Mary F. Gestational diabetes mellitus management with oral hypoglycemic agents. Semin Perinatol 2014; 38(8): 508–515.
3. Ahmadi A, Khalili M, Khatami K, Farsadrooh M, Nahri-Niknafs B. Synthesis and investigating hypoglycemic and hypolipidemic activities of some glibenclamide analogues in rats. Mini-Reviews Med Chem 2014; 14(2): 208–213.
4. Magon N, Seshiah V. Gestational diabetes mellitus: Non insulin management. Indian Journal of Endocrinology and Metabolism 2011; 15(4): 284–293.
5. Balsells M, Garcia-Patterson A, Sola I, Roque M, Gich I, Corcoy R. Glibenclamide, metformin, and insulin for the treatment of gestational diabetes: a systematic review and meta-analysis. BMJ Clinical Research 2015; 350: 1-12.
6. Niopas I, Athanasios, Daftsios**.** A validated high-performance liquid chromatographic method for the determination of glibenclamide in human plasma and its application to pharmacokinetic studies. Journal of Pharmaceutical and Biomedical Analysis 2002; 28: 653–657.
7. Langer O, Conway DL, Berkus MD, Xenakis EMJ, Gonzales O. A comparison of glyburide and insulin in women with gestational diabetes mellitus. The New England Journal of Medicine 2000;343(16):1134–1138.
8. Paithankar HV. HPLC method validation for pharmaceuticals: A review. International Journal of Universal Pharmacy and Bio Sciences 2013;2(4):229-240.
9. Sudha T, Krishna V, Kumar VRR. Development and validation of an analytical method for glyburide and its related compounds in tablet formulation by HPLC-UV. Turkish Journal of Pharmaceutical Sciences 2014; 11(3): 307-316.
10. Atif M, Khalid SH, Onn Kit GL, Sulaiman SAS, Asif M, Chandersekaran A. Development and validation of RP-HPLC-UV method for the determination of glipizide in human plasma. Journal of Young Pharmacists 2013; 5(1):26-29.
11. Ravindra A, Swamy KN, Hima P, Kumar KV. Validated RP-HPLC method for simultaneous estimation of levonorgestrel and ethinylestradiol in combined dosage form. Journal of Scientific and Innovative Research 2013; 2(3):642–50.
12. Latha DM, Ammani K, Kumar P.J . Development and validation of RP-HPLC method for quantitative analysis tolbutamide in pure and pharmaceutical formulations. International Journal of Chemical Sciences 2013; 11(4): 1607-1614.
13. Punugoti RA, Jupally VR. Design and validation of simple UV spectrophotometric method for the assay of glibenclamide in in tablet dosage form. International Journal of Pharmaceutical, Biological and Chemical Sciences 2013; 2(4): 52-56.

Cite this article as:

Ashwini M *et al*. HPLC method development and validation for determination of glibenclamide in pharmaceutical dosage forms. Int. Res. J. Pharm. 2019;10(4):117-120 http://dx.doi.org/10.7897/2230-8407.1004135

Source of support: Nil, Conflict of interest: None Declared