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METHOD DEVELOPMENT AND VALIDATION OF GLIBENCLAMIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY USING UV-VIS SPECTROPHOTOMETRIC METHOD

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ABSTRACT

A simple, sensitive and accurate spectrophotometric method was developed in ultraviolet region for the estimation of Glibenclamide in pure drug, pharmaceutical formulation. Linear response obtained was in the concentration range of 5-30µg/ml with correlation coefficient of 0.999 in acetronitrile: 0.2M NaOH (20:80). Excellent recovery proved that the method was sufficiently accurate. There is no interference from any common pharmaceutical additives and diluents. Results of the analysis were validated by recovery studies according to ICH Q2B guidelines.

Key words: Glibenclamide, UV- Spectrophotometry, recovery, accuracy.

INTRODUCTION

Glibenclamide, chemically 5-chloro-*N*-(4-[*N*-(cyclohexylcarbamoyl) sulfamoyl] phenethyl)-2-methoxybenzamide. Glibenclamide (INN), also known as glyburide (USAN), a second-generation sulfonylurea antidiabetic[1] agent, appears to lower the blood glucose acutely by stimulating the release of insulin from the pancreas, an effect dependent upon functioning beta cells in the pancreatic islets[1]. With chronic administration in Type II diabetic patients, the blood glucose lowering effect persists despite a gradual decline in the insulin secretary response to the

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drug. Sulfonylureas[1] such as glibenclamide bind to ATP-sensitive potassium channels on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the membrane. Depolarization stimulates calcium ion influx through voltage-sensitive calcium channels, raising intracellular concentrations of calcium ions, which induces the secretion, or exocytosis, of insulin[1]. Estimation of glibenclamide from human serum by using HPLC was been reported[2]. Literature survey tells about the simultaneous estimation of glibenclamide, glipizide and metformin by using ultra fast HPLC[3] and also tells about the simultaneous estimation of six anti-diabetic[4] drugs— glibenclamide, gliclazide, glipizide, pioglitazone, repaglinide and rosiglitazone by using HPLC[4]. A number of assay methods for use in pharmacokinetic studies are available for determination of glibenclamide in biological specimen[5, 6,7,8,9].

MATERIALS AND METHODS

Glibenclamide sample was supplied by Cadila Pharmaceutical Ltd. Ahmedabad, India as a gift sample. The solvent used was acetonitrile and NaOH was of AR grade, purchased from SD Fine Chemicals Limited, India. Spectral and absorbance measurements were made on a UV-Visible spectrophotometer (Shimadzu UV–1800) model with 10 mm matched pair of quartz cell and spectral bandwidth of ±2nm.

EXPERIMENTAL:-

Determination of λ max:-

Accurately weighed 10 mg of Glibenclamide is transferred into a 100 ml volumetric flask and dissolved in 20 ml of acetonitrile. It was then sonicated for 10 min, and made up to the mark with 0.2M NaOH to give a stock solution having 100 µg/ml concentrations. This solution was subjected to scanning between 200-400 nm and absorption maxima at 229 nm was determined. The effect of dilution maxima was studied by diluting the above solution to 20 µg/ml and scanned from 200-400 nm.

Standard solutions:-

Accurately weighed 100 mg of Glibenclamide is transferred into a 100 ml volumetric flask. It was dissolved in 20 ml of acetonitrile and sonicated for 10 min, and made up to the mark with 0.2M NaOH to give a stock solution having 1000 µg/ml concentrations.

Working standard solution:

10 ml of stock solution was further diluted to 100ml with 0.2M NaOH to obtain a working standard solution containing 100 µg/ml.

METHOD VALIDATION[10]:

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The method was validated for different parameters like Linearity, Accuracy, Precision, Specificity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).

Linearity and Calibration:-

The aliquots working standard solution were diluted serially with 0.2M NaOH to obtain the range 5-30 µg/ml. a calibration curve for Glibenclamide was obtained by measuring the absorbance at the λ_{max} of 229 nm. Statistical parameters like slope, intercept, coefficient of correlation, standard deviation were determined.

Analysis of marketed tablet formulation:

Determine the content of Glibenclamide in conventional tablets (label claim: 5 mg Glibenclamide per tablet), twenty tablets were weighed. Their mean weight was determined, they were finely powdered, and powder equivalent to 100 mg of Glibenclamide was weighed and transferred into a 100 ml volumetric flask containing 20 ml acetonitrile, sonicated for 10 min and the resulting sample solution was then filtered through Whattman filter paper (No. 41). The filtrate was further diluted with 0.2M NaOH to obtain the final concentration of 1000 µg/ml. Appropriate dilutions of Glibenclamide were scanned over the range of 200-400 nm and the absorbance at wavelength 229 nm was measured. From calibration curve, the final drug concentration in tablet was calculated.

Recovery studies:

Recovery studies were performed to judge accuracy of the method. 1ml of standard solution (100 µg/ml) was taken in three 10 ml volumetric flask and to it add 0.8, 1.0 & 1.2ml (*i.e.* 80%, 100%, 120%) of working standard solution (100 µg/ml) added respectively and made the volume upto the mark. The respective absorbance at 229nm was recorded against the blank. The amount of added concentration was determined from the obtained absorbance values and percent recovery was determined for each formulation.

RESULTS AND DISCUSSION

The UV scan of standard solution between 200-400 nm showed the absorption maxima at 229 nm, shown in **Figure 1.** The Beer's law was verified from the calibration curve by plotting a graph of concentration Vs absorbance. The plot shown in **Figure 2.** Regression analysis showed

very good correlation. The calibration plot revealed zero intercept which is clear by the regression analysis equation Y= m X+C (where Y is absorbance, m is the slope and X is the conc.) the results obtained are depicted in Table 1,2,3,4. No significant variations were observed on interday and intraday analysis.

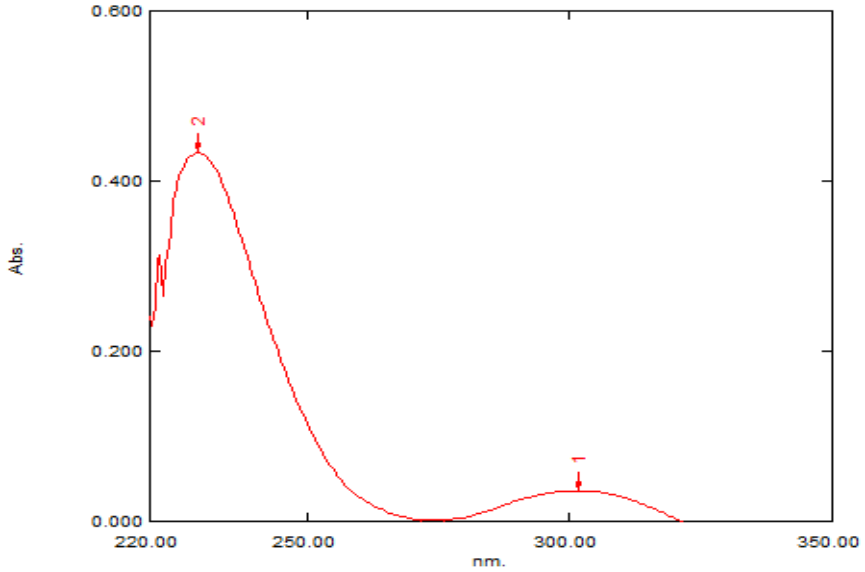

Figure 1: Spectrum of Glibenclamide at wavelength 220 to 350 nm

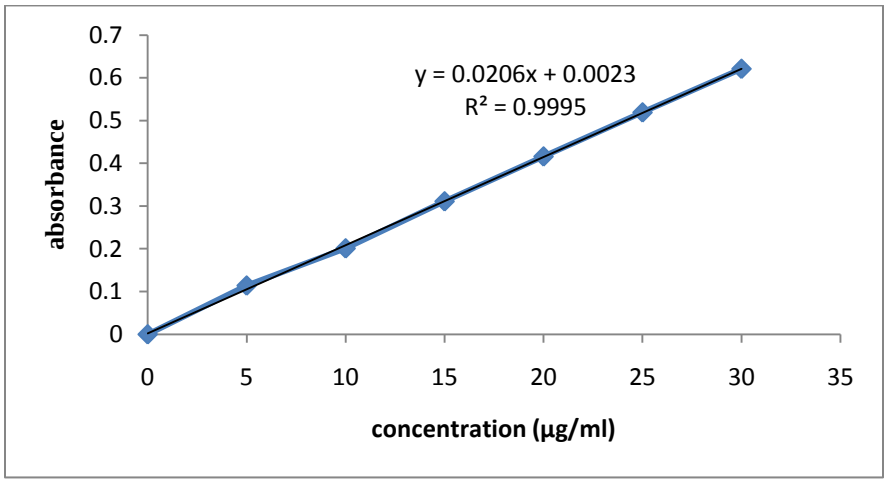

Figure 2: Calibration curve of Glibenclamide showing linearity relationship

Table 1: Linearity regression data for Glibenclamide

Parameters	Value for Glibenclamide
Beer's law limit (µg/ml)	5-30 µg /ml
Correlation coefficient	0.999
Regression equation (Y*)	y = 0.020x + 0.002
Slope	0.020
Intercept (A)	0.002

Table 2: Results of analysis of laboratory samples

Label claim (mg/tab)	% Concentration estimated*(Mean ± % R.S.D.)
5 mg	4.98

* Average of nine determinations; R.S.D., Relative Standard Deviation

Table 3: Recovery data for Glibenclamide

Level added (%)	Recovery (%)*	SD
80	100.42	0.7824
100	99.61	0.2916
120	99.98	0.1944

Table 4: Limit of detection and limit of quantitation for Glibenclamide

L.O.D (µg/ml)	L.O.Q(µg/ml)
0.1927	0.5840

The spectrum of Glibenclamide in 0.2M NaOH showed the absorption maxima at 229nm. No effect of dilution was observed on the maxima which confirmed the maxima at 229nm. The statistical analysis of data obtained for the calibration curve of Glibenclamide in the high level of precision for the proposed method. The coefficient of correlation was highly significant. The linearity range was observed between 5-30 µg/ml. the plot clearly showed a straight line passing through origin. The estimated method was validated by % RSD, accuracy, precision of the methods.

CONCLUSION

From the above result and discussion the method describe in this work for the determination of Glibenclamide from tablet formulation is simple, accurate, sensitive and economical. The proposed method utilizes inexpensive solvent. The proposed method could be applied for routine analysis in quality control laboratories.

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