Research Article

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Development and Validation of UV Spectrophotometric method for estimation of Glibenclamide in bulk and Pharmaceutical dosage forms

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ABSTRACT

A selective, simple, accurate and reproducible spectrophotometric method has been developed for the estimation of Glibenclamide in bulk and pharmaceutical formulation. The drug obeyed the Beer's law and showed good correlation. It showed absorption maxima at 300nm in methanol. The developed method was validated with respect to linearity, accuracy and precision. The linearity was observed between 10-100µg/ml having line equation Y=0.0069X + 0.0209 with correlation coefficient of 0.9988. Results of the analysis were validated statistically and by recovery study.

KEY WORDS: UV-Vis Spectrophotometer, Glibenclamide(GLB), Recovery, λmax.

INTRODUCTION:

5-chloro-N-[2-(4-{[(cyclohexylcarbamoyl)amino]sulfonyl} phenyl) ethyl]-2-methoxybenzamide is the chemical name of Glibenclamide, widely used as an antidiabetic(1). Glibenclamide, also known as glyburide, a second-generation sulfonylurea antidiabetic 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. With chronic administration in Type II diabetic patients, the blood glucose lowering effect persists despite a gradual decline in the insulin secretory response to the drug. Extrapancreatic effects may be involved in the mechanism of action of oral sulfonyl-urea hypoglycemic drugs. Sulfonylureas 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. Several methods have been published for the determination of GLB, either in pharmaceutical preparations and biological fluids. These methods include spectrophotometry (11),and HPLC(2-9).There is a need to develop and validate a new simple, rapid, reliable and precise UV spectrophotometric method for analysis of GLB in bulk and tablet formulation. Suitable statistical tests were performed on validation data (10,12).

MATERIALS AND METHODS:

Instrument Used:

A Model:UV-Vis spectrophotometer 1700

Make:Shimadzu,Kyoto,Japan
Scan speed:40nm/min

B. Bath Sonicator

Reagents and Solutions:

All the reagents used in this assay were of analytical grade . Tablets of GLB were purchased.

EXPERIMENTAL:

Determination of λmax:

Weighed amount of GLB was dissolved in methanol to obtain a 100µg/ml solution. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.The effect of dilution on absorption maxima was studied by diluting the above stock solution to 40µg/ml and scanned from 200-400nm.

Preparation of standard stock solution:

Standard drug solution of GLB was prepared by dissolving 10 mg GLB in 100 ml methanol to obtain stock solution of 100 µg/ml concentration.

Preparation of calibration curve:

Calibration curve was prepared in methanol at λ max 300 using UV-Vis spectrophotometer Model 1700.For this stock solution of 100 µg/ml was prepared. Serial dilution of 10, 20, 40, 60, 80,100µg/ml were prepared and absorbance was taken at λmax 300.Averages of such 6 sets of values were taken for calibration curve, and solution were scanned in the range of 200-400 nm against blank. The calibration curve was plotted as shown in fig 2.and the data shown in Table no.2. The optical characteristics are summarized in Table no. 1 and the overlain spectrum of glibenclamide shown in fig.1.

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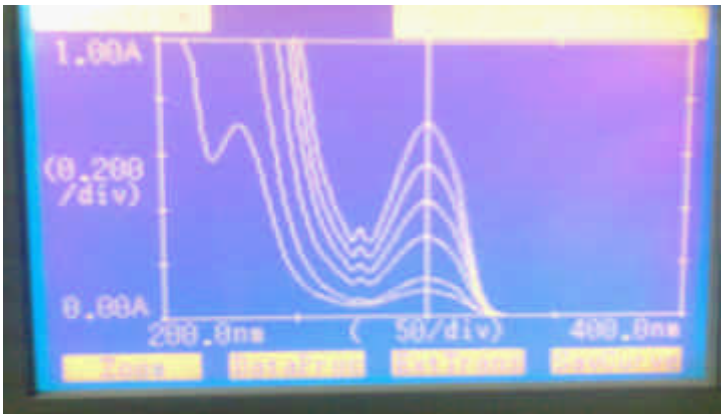

Fig . 1 Overlain spectrum of Glibenclamide

Table1. Optical parameters for glibenclamide

Sr. no	Parameters	Data
1	λ-Max	300nm
2	Linearity	10-100ug/ml
3	Regression equation	Y=0.0069X+0.0209
4	Correlation coefficient	R2 = 0.9988
5	Slop	0.0069
6	Intercept	0.0209
7	LOD	1.016688 µg/mL
8	LOQ	3.080874 µg/mL

Table 2. Calibration data

Concentration	Absorbance	SD	RSD
10	0.099	0.001549	1.564842
20	0.1455	0.001517	1.04232
40	0.302333	0.002338	0.773349
60	0.431333	0.003882	0.975655
80	0.5655	0.002258	0.399349
100	0.713333	0.001211	0.169775

Table 3. Accuracy data for analysis of glibenclamide(recovery studies)

Amount of sample taken (mg/ml)	Amount of standard added (mg/ml)	Total amount Recovered (mg/ml)	SD	% Recovery
60	0	59.466	0.305505	99.111
60	30	89.82	0.230651	99.8
60	60	120.766	0.723418	100.6389
60	90	149.693	0.621718	99.7955

Table.4.Repeatabality data for glibenclamide

Conc (mg/ml)	Interday Absorbance	SD	%RSD	Intraday Absorbance	SD	RSD
40	0.3066	0.003215	1.048223	0.304	0.002	0.657895
60	0.4393	0.003055	0.695383	0.43433	0.003215	0.740111
80	0.57	0.001732	0.303869	0.56633	0.003215	0.567607

Assay:

Ten Tablets each containing of 5 mg of GLB was weighed and powdered. Powder equivalent to 100 mg of GLB was transferred into 100 ml volumetric flask dissolved in methanol. The solution was then filtered through Whattman filter paper No 40 (0.45 micron).Aliquots of the sample were removed and diluted to 10 ml of methanol to obtain strengths of 40µg/ml determined at the respective absorbance of 300nm against methanol as a blank.(Table5)

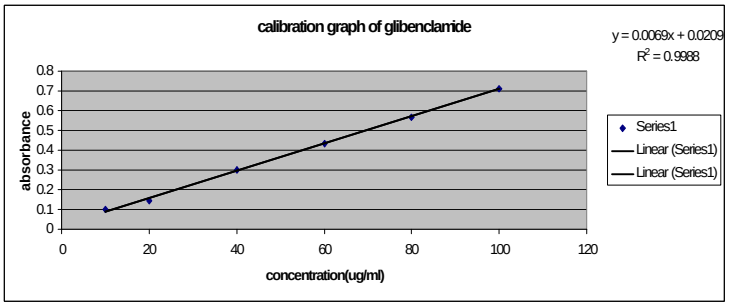

Fig . 2 Calibration curve of Glibenclamide

Table 5. Determination of glibenclamide marketed formulation.

Tablet code	Label claim (mg/tab)	Amount found (mg)	%
GLIBANI	5	5.05	101
GLIBANII	5	4.96	99.2

Recovery studies:

Recovery studies were performed to judge the accuracy of the method. Recovery studies were carried out by adding a known quantity of pure drug to the pre-analyzed formulation and the proposed method was followed. From the amount of drug found, percentage recovery was calculated.Recovery study was carried out by addition of standard drug to the sample at 3 different concentration levels.(Table 3)

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD and LOQ of GLB were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines 7. The LOD and LOQ were found to be as inTable No 1. Limit of detection (LOD) and limit of quantification (LOQ) were calculated by Eq. (1)LOD =3.3δ/ *s* and (2) LOQ =10δ/ *s* respectively, where δ is the standard deviation of blank and *s* is slope.

RESULTS:

The UV scan of standard solution between 200 – 400 nm showed the absorption maxima at 300nm, shown in fig 1. The Beer's law was verified from the calibration curve by plotting a graph of concentration vs. absorbance. The plot is shown in fig 2. Regression analysis showed very good correlation. The results thus obtained are depicted in table 1 .The results of analysis for recovery studies for marketed formulations were studied and are shown in table 3 .

DISCUSSION:

The spectrum of GLB in methanol showed the absorption maxima at 300nm. No effect of dilution was observed on the maxima, which confirmed the maxima at 300nm. The statistical analysis of data ob-

tained for the calibration curve of GLB in pure solution indicated a high level of precision for the proposed method. The coefficient of correlation was highly significant. The linearity range was observed between 10 – 100µg/ml. The plot clearly showed a straight line passing through origin equationY=0.0069X + 0.0209 with correlation coefficient of 0.9988. The assay method was validated by low values of standard deviation and standard error, indicating accuracy and precision(Table4) of the methods. Excellent recovery studies further proves the accuracy of the method.

CONCLUSION:

It can be concluded that the proposed method is simple, rapid, accurate, precise, economic and reproducible for UV spectrophotometric estimation of GLB from pharmaceutical formulation. This method can be successfully applied for routine estimation of GLB in bulk, pharmaceutical dosage form.

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