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Development and Validation of a Spectrophotometric Method for Glibenclamide in Bulk and Tablet Dosage Forms

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Abstract: Two simple spectrophotometric methods have been developed for estimation of Glibenclamide from tablet dosage form. First method, Area under curve method, area under curve in the range of 295.0-310.0 nm was selected for the analysis. Second method is First order derivative spectroscopy, the absorbance was measured at λmax=296.50 nm, λmin=308.3 nm & Zero cross=301.0nm. Linearity for detector response was observed in the concentration range of 40-90 µg/ml. The accuracy and precision of the methods were determined and validated statically. All the methods showed good reproducibility and recovery with % RSD less than 2. The proposed methods were found to be rapid, specific, precise and accurate and can be successfully applied for the routine analysis.

Keywords: Accuracy, Precision, % Recovery, Glibenclamide, First order derivative spectroscopy, Area under curve method.

Introduction:

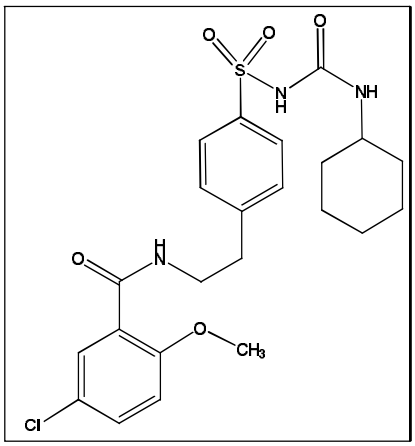

Figure No.1- Chemical Structure of Glibenclamide

Glibenclamide (Fig.1) is chemically1-{4-[2-(5–Chloro-2–methoxybenzamido)ethyl]benzene sulphonyl}-3-cyclohexylurea.[1] It is a compoundthat acts by inhibiting ATP-sensitive potassium channels in

pancreatic β-cells causing cellmembrane depolarisation that increase intracellular calcium in the beta cell which stimulates the insulin release. It is oral hypoglycemic drug second generation sulphonyl-ureas drug widely used in treatment of Type-2 diabetic patient. [1, 2, 3]

A detailed survey of literature revealed the estimation of Glibenclamideby RP-HPLC [4], spectrophotometer [5], LCMS [6]. Several techniques have been reported for assay by quantitative determination of Glibenclamide in biological fluids which high performance liquid chromatography (HPLC), LC-MS in plasma and UV-Spectropthometric techniques in dosage forms. [4, 5, 6]

This study describes two new highly sensitive, rapid, simple, accurate, precise, reproducible, economical visible spectrophotometric methods for the determination of Glibenclamide in pure and in tablets. [2, 7]

Materials and Methods:

Adouble-beamUV-Visible spectrophotometer, model UV-1800 (Shimadzu, Japan) having two matched cells with1cm lightpath.A Citizen analytical balance (Sartorius) was used for weighing the samples.Glibenclamide pure drug was obtained from SANOFI INDIA LTD as gift sample with 99.9%w/w assay value and was used without furthe rpurification.All other chemicals and solvents used were of analyticalgrade.

Preparation of standard stock solutions:

Standard stock solutions of Glibenclamide wasprepared by dissolving 10 mg of drug in10ml of methanol to get standard stock solution of 1000µg/ml respectively and 1ml was pipette out and further volume was made upto 10ml with methanol to obtain concentration of 100µg/ml. Further dilutions were made in distilledwater from stock solution to get concentrations of 40-90 µg/ml of Glibenclamide.

Determination of Absorption Maxima :

Accurately weighed Glibenclamide (10 mg) was transferred to a 10 Ml volumetric flask, dissolved in methanol and diluted to10mLwith water. The solution (1mL) was transferred to a 10 mL volumetric flask and diluted up to the mark with water to obtain final solution of Glibenclamide (100 µg/mL). The working standard stock solutions of Glibenclamide was scanned in the range of 200 to 400 nm against methanol as a blank. The absorbance of each solution was measured at the wavelength 301.70 nm. (Fig.2)

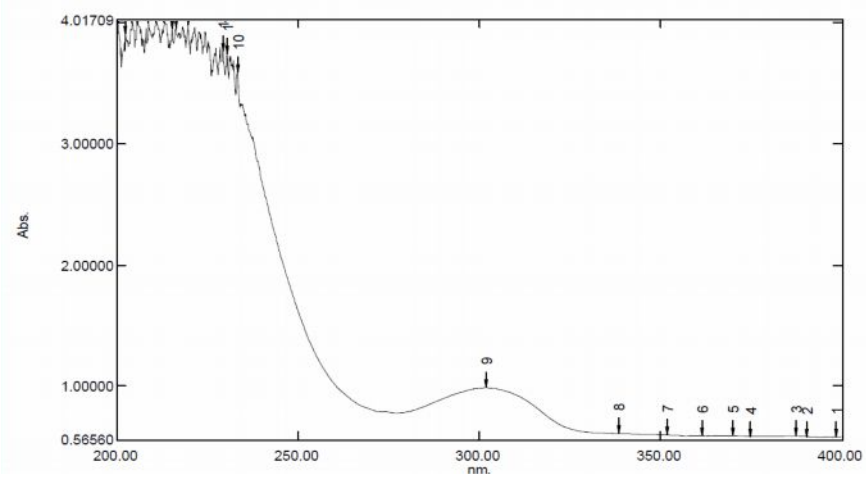

Figure No. 2- Determination of λ_{max} of Glibenclamide std. stock solution

Area under curve method (Method I):

From the spectra of drug (Fig. 3), area under the curve in the range of 295.0-310.0 nm was selected for the analysis. The calibration curves for Glibenclamide was prepared in the concentration range of 40-90 µg/mL

at their respective AUC range. The 'X' values of the drugs were determined for the drug at the selected AUC range. The 'X' is the ratio of area under the curve at selected wavelength ranges with the concentration of component in gm/lit. These 'X' values were the mean of six independent determinations.

Where,

First Order Derivative Spectroscopy (Method II):

In this method solutions of Glibenclamide (40-90 µg/ml) were prepared separately by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. The absorption spectra thus obtained were derivatized from first to fourth order. First order derivative spectra were selected for analysis of drug. From the spectra of drug (Fig. 4), the absorbance was measured at λ_{max}=296.50 nm, λ_{min}=308.3 nm & Zero cross=301.0 nm, amplitude difference was measured for the respective concentration of standard and was plotted against concentration and regression equation was calculated.

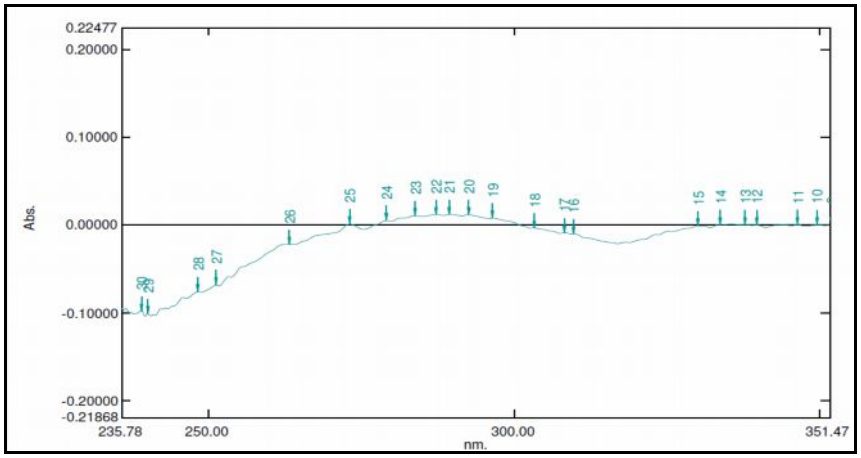

Figure No. 4- First Derivative Spectra of Glibenclamide std. stock solution

Application of the proposed methods for the determination of Glibenclamide in commercial formulation.

The powder of 20capsules was weighed, mixed and accurately a quantity of the powder equivalent to about 5mg of Glibenclamide is transferred into100mL measuring flask. The solution was filtered through Whatman filter paper No.41 and the residue was washed thoroughly with methanol. After rejecting first few ml, different concentrations of tablet sample were prepared by serial dilution technique with distilled water. Absorbance of sample solutions were recorded at 301.70 nm

For Method-I, the concentration of Glibenclamide was determined by measuring area under curve in the range of 295.0-310.0 nm and values were substituted in the respective formula to obtain concentrations. The analysis procedure was repeated for 6 times with Tablet formulations. The concentration of Glibenclamide was determined by measuring at λmax=296.50nm, λmin=308.3 nm & Zerocross=301.0 nm. The results of the Tablet analysis was calculated against the calibration curve in quantitation mode (Method II).The results are reportedin Table.1

Table No. 1: Results of Analysis of Tablet Formulation

Method	Capsule content	Label claim (mg/tab)	Amount Found*		±SD	% RSD
			(in mg)	(In %)		
I	Glibenclamide	5	4.99	99.96	0.00089	0.00137
II			4.99	99.93	0.0068	1.05

*denotes n=6, average of six determinations

Validation

The methods were validated with respect to linearity, accuracy, precision and selectivity.

Accuracy

Recovery studies were carried out to ascertain the accuracy of the proposed methods by Standard addition method at three different levels 80%, 100% & 120% (Table 2). The mean percent recovery for Glibenclamide by all the two methods was found in the range of 99.77 % to 99.98%.

Table No. 2: Result of Recovery Studies

Level of Recovery	Drug	Amt. of drug added (in µg)	Amt. of drug std. added (in µg)	Method I		Method II	
				% Recovery	SD	% Recovery	SD
80%	Glibenclamide	48	47.89	99.77	0.0306	99.81	0.00412
100%		60	59.99	99.98	0.00183	99.98	0.000265
120%		72	71.93	99.90	0.0028	99.92	0.00238

Linearity

The six-point calibration curves that were constructed were linear over the selected concentration range for Glibenclamide ranging between 40-90 µg/mL. Each and every concentration was repeated 3 times. According to the experimental conditions previously described the assay was performed. The linearity of the calibration graphs and as well the adherence of the system to Beer's law were validated successfully by the high value of the correlation coefficient (R^2) and the intercept value (c).

Precision

Result is expressed in % RSD for intraday and interday precision (Table 3). The reproducibility of the proposed method was determined by performing the Tablet assay on same day (Intraday assay precision) at different time intervals (morning, afternoon and evening) and on three different days (Interday precision). Percent RSD for Intraday assay precision was found to be 0.0015 in area under the curve method and 0.159 in First derivative spectrophotometric method. Interday assay precision was found to be 0.00289 in area under the curve method and 0.00344 in First derivative spectrophotometric method.

Table No. 3: Results of Intermediate Precision

Day	Method I	Method II
	% Label claim estimated* (Mean ± % RSD)	% Label claim estimated* (Mean ± % RSD)
Intra day	4.95 ± 0.0015	4.95 ± 0.159
Inter day	4.92 ± 0.00289	4.93 ± 0.00344

Results and Discussion-

Glibenclamide in methanol water (50:50) exhibited λmax at 301.7nm. The Beer's law was obeyed to the method in the concentration range of 30-80µg/ml respectively. The optical characteristics such as Beer's law limits (mg/ml),Molar extinction coefficient (L/mol.cm), Regression equation(y), Correlation coefficient calculated from five-six measurements containing 3/4th of the amount of upper Beer's law limits and was calculated for Glibenclamide.

Conclusion-

The proposed method was found to be simple, sensitive,accurate, precise, and reproduciblecan be used for routine quality control analysis of Glibenclamide in bulk andin pharmaceutical dosage forms.

Table No. 4: Validation parameters for UV-Spectroscopic methods

Parameters	Method I	Method II
Linearity range (µg/mL)	40-90 µg/mL	40-90 µg/mL
Correlation coefficient (R^2)	0.9987	0.9701
Sandell's sensitivity (µg/cm^2 /0.001)		
Slope	0.0153	0.012
Intercept	- 0.269	- 0.0402

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