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Research Article

SIMULTANEOUS DETERMINATION OF METFORMIN AND GLIBENCLAMIDE BY ULTRA-VIOLET SPECTROPHOTOMETRY

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ABSTRACT

This research deals with simultaneous determination of Glibenclamide and Metformin Hydrochloride in combined dosage form. The method utilized simultaneous equation method for analysis using 0.1 N HCL as a solvent. The two wavelengths 300 nm and 233 nm were selected for determination of Glibenclamide and Metformin Hydrochloride respectively. Beer's law was obeyed in concentration range of 10-60µg/ml and 2-12µg/ml for Glibenclamide and Metformin Hydrochloride respectively. The recovery studies confirms the accuracy of the projected method and the results were validated as per ICH guidelines. The proposed method is simple and rapid, can be employed for determination of Metformin Hydrochloride and Glibenclamide in pharmaceutical dosage form.

Keywords: Glibenclamide, Metformin Hydrochloride, Simultaneous equation

INTRODUCTION

Metformin HCl is an antidiabetic agent belongs to biguanide class, chemically it is known as NN, Dimethylimido dicarbonimidediamide. It is a first choice anti-diabetic for the treatment of type 2 diabetic especially in case of overweight patients. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose and improves intestinal sensitivity by increasing peripheral glucose uptake and utilization. Whereas chemically, Glibenclamide is identified as 5 Chloro-N-[2-[4-[[[cyclohexylamine] carbonyl]-amino] sulphonyl] phenyl] ethyl -2-methoxybenzamide with a molecular weight of 490.62. Glibenclamide binds to ATP-sensitive potassium channel receptors 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 concentration of calcium ions, which induces the secretion, or exocytosis, of insulin. Metformin HCl have high solubility and low permeability whereas Glibenclamide have low solubility and high permeability and are classified under class III and II biopharmaceutical classification system (BCS).[1]

Rationale of Glibenclamide and Metformin hydrochloride suggests the use of combined formulations of medicaments capable of finding a remedy for both the deficiency in insulin secretion and the insulin resistance condition. In unexpected prevalence of hyperglycemia, a dose of 5mg of glibenclamide is required to reduce the hyperglycemic effect and 500mg of metformin hydrochloride is required to sustain the normal glycemic level. [2]

Several assay method have been described for quantitative resolution of Glibenclamide in biological fluid, these methods involves the procedure based on HPLC, Fluorometry, radioimmunoassay and gas chromatography[3-9]. A number of reports maintain the evaluation of the drug in these dosage form, such methods include micellar electrokinetic, capillary chromatography, RP HPLC, TLC UV spectrophotometry, derivative spectrophotometry and colorimetry. Few UV Spectrophotometric approaches HPLC and ion-pair HPLC procedures have been reported for the estimation of Metformin.

Need of the Study

Glibenclamide is used in different combination with Metformin hydrochloride. In these combinations qualitative determination of each ingredient without the interference of other active or inactive ingredient is quiet important. Most of cases these compounds are detected on HPLC equipped with a UV detector. In the marketed formulation of Metformin HCl and Glibenclamide the difference in concentration is quiet significant as Glibenclamaide is present 2.5or 5mg on the other hand Metformin HCl is 250 or 500mg. Furthermore the wavelength for maximum absorbance for both compounds is almost same as 229 nm for glibenclamide and 233 for Metformin HCl. This difference in concentration and same λmax make it quite difficult to get comparable peaks of Metformin HCl and glimepiride in same chromatogram. The aim of this research is the development of a procedure for the simultaneous determination of glibenclamide and Metformin HCl in a biological fluid which can be used for assay and release profile studies of the formulations containing Metformin hydrochloride and Glibenclamide.

MATERIALS AND METHODS

Instruments

The absorption spectra of the solutions were recorded over the range of 200-400 nm using Agilent UV visible Spectrophotometer.

Chemicals

The working standards of Glibenclamide and Metformin were obtained from Cipla Ltd as a gift sample. Methanol and HCl used were obtained from Central drug house pvt. Ltd. Delhi.

Selection of Solvent System

In this present study the main objective is to determine the gastroretentive formulation composed of Metformin hydrochloride and Glibenclamide. As per the concept of floating, the formulation should retain their integrity and release the drug at a constant rate in stomach environment and as we know pH 1.2 (average pH of stomach) is considered in most of the cases, as pH of choice for evaluation of GRDDS. 0.1N HCl poses same the pH value as that of stomach environment. The solubility profile of Metformin hydrochloride and Glibenclamide are very different from each other. Metformin hydrochloride is freely soluble in 0.1 N HCl whereas glibenclamide is poorly soluble. As per the concept of simultaneous determination we have to use the same solvent for both the drugs. To achieve solubility of glibenclamide in 0.1 N HCl concept of cosolvency using methanol as cosolvent was utilized.

Preparation of stock solution

i) UV- Spectrum of pure Glibenclamide was observed in methanolic 0.1 N HCl (pH 1.2) as a medium. Drug (10 mg) was dissolved in 100 ml 0.1 N HCl to obtain the stock solution of concentration 100 µg/mL.
ii) UV- Spectrum of pure Metformin HCl was observed in 0.1 N HCl (pH 1.2) as a medium. Drug (10 mg) was dissolved in 100 ml 0.1 N HCl to obtain the stock solution of concentration 100 µg/mL.

Selection of absorption maxima

Ten ml of Glibenclamide and Metformin was taken from their standard stock solution and was transferred to 100 ml volumetric flask, dissolved in 0.1 N HCL and volume was made up to the mark to obtain concentration 10 µg/ml of both the drugs. Drugs solution was scanned separately between 200-400 nm and two wavelengths 300 nm (Abs.maxima for Glibenclamide) and 233 nm (Abs.maxima for Metformin HCL) were selected for the study .Figure 1 and 2 show absorption maxima of both the drugs.

Simultaneous Equation

Series of drug concentrations were prepared from stock solutions using 0.1 N HCL as a solvent. The absorbance of these solutions was measured at 300 nm and 233 nm for Glibenclamide and Metformin, respectively and calibration curves were plotted at selected wavelengths. Two simultaneous equation (in two variables CMET and CGLB) were framed based upon the fact that at 300 and 233 nm the absorbance of the mixture is the sum of the individual absorbances of Glibenclamide and Metformin.[10-11]

At λ_1

$A_1 = a_{x1}bc_x + a_{y1}bc_y$ (1)

At λ_2

$A_2 = a_{x2}bc_x + a_{y2}bc_y$ (2)

Where; $\lambda 1$= 300 nm, $\lambda 2$= 233 nm

The absorptivities of Glibenclamide at 300 and 233 nm, a_{x1} and a_{x2} respectively

The absorptivities of Metformin at 300 and 233 nm, a_{y1} and a_{y2} respectively

The absorbances of the diluted sample at 300 and 233 nm, A1 and A2 respectively

b is path length (here b=1cm), c_x is the concentration of Glibenclamide, c_y is the concentration of Metformin

Now,

Rearranging the equation no (2)

$$c_y = \frac{A2 - a_{x2}c_x}{a_{y2}}$$

Substituting for c_x in equation (1) and rearranging gives

$$c_x = \frac{A_2 a_{y1} - A_1 a_{y2}}{a_{x2}a_{y1} - a_{x1}a_{y2}}$$

$$c_y = \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{x2}a_{y1} - a_{x1}a_{y2}}$$

Now,

Substituting the values in the above equation

$c_x = A_2(214.966) - A_1(813.250)\}/-57033.0125$

$c_y = A_1(48.753) - A_2(83.016)\}/-57033.0125$

Preparation and analysis of tablet formulations

The projected method was also applied to determine Metformin Hydrochloride and Glibenclamide in bilayer tablets, for this twenty tablets (containing 5 mg of GLB and 500 mg of MET) were weighed and triturated to fine powder. Correctly weighed 245mg of pure Glibenclamide was added to above powdered sample to make the concentration of Glibenclamide in linearity range .With this incorporation, the ratio of both drugs in samples was brought to 1:2. A quantity of sample equivalent to 250 mg of Glibenclamide and 500 mg of Metformin HCL was transferred into 100 ml volumetric flask containing 50 ml of methanolic 0.1N HCL sonicated for 15 min, the volume was made up to the mark and filtered through Whatman filter paper (No.41).0.1 ml of this solution was transferred to 100 ml volumetric flaks, dissolved and volume was adjusted to mark. The absorbance of the solutions was recorded at 300nm and 233nm against blank. The concentrations of two drugs in sample were determined by using equations state above**.** The results are reported in the Table 5.

Validation

Recovery studies were evaluated by testing three concentrations i.e. 80, 100 and 120% of the label claim of the tablet formulation as per ICH guidelines. Triplicate measurements were done for each prepared solutions [12-13]. The results of the recovery studies were also validated statistically. The results of recovery studies are given in table no 6.

Precision

Precision of the methods was studied as intra-day, inter-day and repeatability. Intra-day study was performed by analyzing, the three different concentration of drug for three times in the same day. Inter-day precision was performed by analyzing three different concentration of the drug for three days in a week. Repeatability was performed by analyzing same concentration of drugs for six times. The results are shown in table 7.

RESULT AND DISCUSSION

The UV spectrum of both the drugs showed two wavelengths 300 nm (λ max for GLB) and 233 nm (λ max for MET) in respective solvent. Beer's law was obeyed in concentration range of 10-60µg/ml and 2-12µg/ml for Glibenclamide and Metformin Hydrochloride respectively. The amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy and precision of the study were established at three different concentration levels. Recovery experiments showed good results.

Both the methods were found to be precise as indicated by the repeatability, inter-day, intra-day analysis, showing %RSD in the acceptable range. All statistical data proves validity of the methods and can be used for routine analysis of Gastro retentive pharmaceutical formulations containing both these drugs in 0.1 N HCl which poses same pH value as stomach environment while in the earlier efforts the simultaneous determination of these both was performed in organic solvents like ethanol, methanol, chloroform etc. whereas in the present study the determination was carried out in solvent which mimics the biological environment of stomach.

Table 1: **Standard curve of Metformin HCL at 233nm in 0.1N HCL**

Concentration	Absorbance	Absorptivity	$E_{1\%}$
2	0.1572	0.076	760
4	0.3251	0.0812	812
6	0.5016	0.0836	836
8	0.6790	0.0845	845
10	0.8464	0.0802	802
12	0.9828	0.0812	813

Table 2: **Standard curve in Glibenclamide at 300nm in 0.1N HCL**

Concentration	Absorbance	Absorptivity	$E_{1\%}$
10	0.0893	0.00893	89.3
20	0.1772	0.00866	86.6
30	0.2443	0.00814	81.4
40	0.3201	0.00800	80
50	0.4032	0.00806	80.6
60	0.4816	0.00802	80.2

Table 3: **Standard curve in Metformin at 300nm in 0.1N HCL**

Concentration	Absorbance	Absorptivity	$E_{1\%}$
2	0.0101	0.00505	50.5
4	0.0518	0.01295	129.5
6	0.1450	0.02416	241.6
8	0.2190	0.02737	273.7
10	0.2949	0.02949	294.9
12	0.3549	0.02995	299.5

Table 4: **Standard curve in Glibenclamide at 233 nm in 0.1N HCL**

Concentration	Absorbance	Absorptivity	$E_{1\%}$
10	0.0512	0.00512	51.20
20	0.1011	0.005055	50.55
30	0.1560	0.0052	52
40	0.1903	0.004757	47.57
50	0.2310	0.00462	46.20
60	0.2704	0.00450	45

Table 5: **Tablet Formulation Analysis**

Drug (n=6)	% Amount found ±SD
Glibenclamide	99.23±0.29
Metformin	99.57±0.12

Table 6: **Recovery Studies**

Amount present (mg) (n=6)	Amount of standard added (µg/ml)	Amount Recovered (µg/ml)	% Amount recovered	% RSD
Glibenclamide 5 µg/ml	4	8.90	98.89	0.15
	5	9.910	99.1	0.76
	6	10.956	99.6	0.26
Metformin 10 µg/ml	8	17.90	99.4	0.86
	10	19.56	97.8	0.72
	12	21.91	99.5	0.47

Table 7: **Precision**

Parameters	Glibenclamide	Metformin HCL
Precision(%RSD)		
Intraday(n=3)	0.68	0.79
Interday(n=3)	0.84	0.96

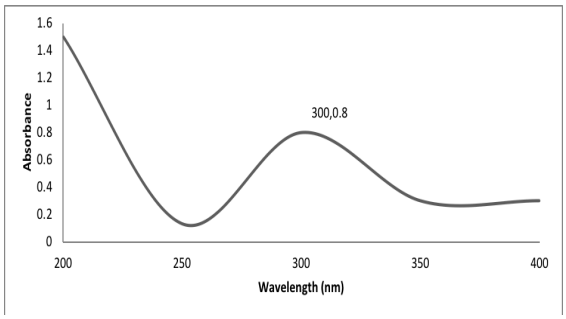

Fig 1: Absorption maxima of the Glibenclamide

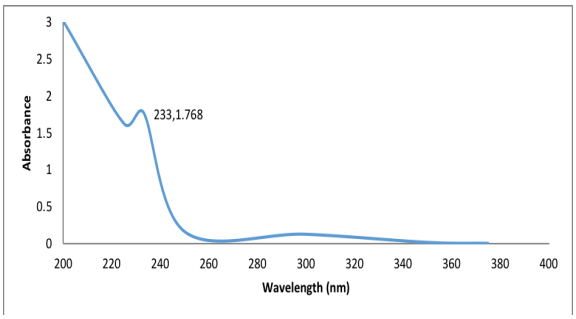

Fig 2: Absorption maxima of the Metformin

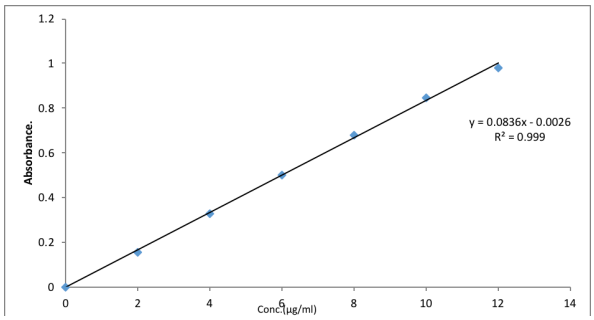

Fig 3: Calibration curve of Metformin at 233 nm in 0.1N HCL

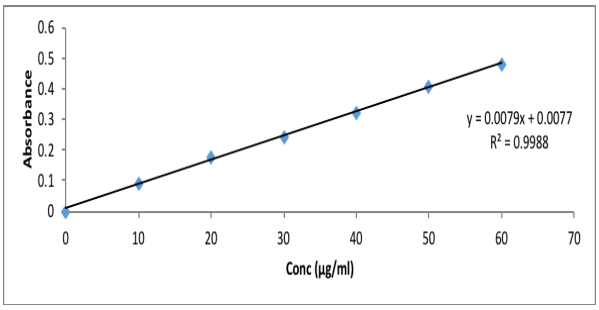

Fig 4: Calibration curve of Glibenclamide at 300nm in 0.1N HCL

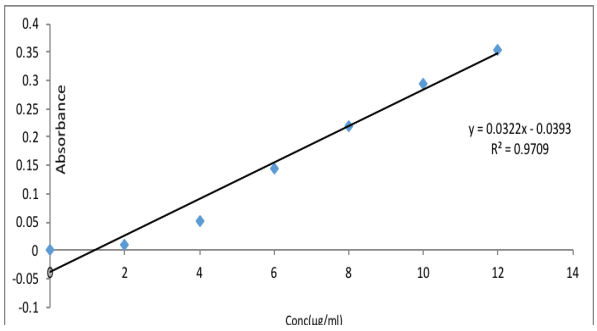

Fig 5: Calibration curve of Metformin at 300nm in 0.1N HCL

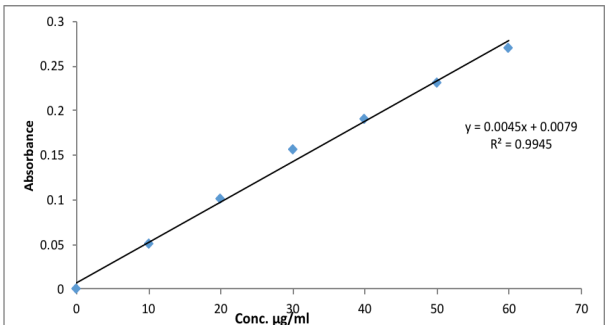

Fig 6: Calibration curve of Glibenclamide at 233 nm in 0.1N HCL

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