

# Pharmacokinetic studies of metformin and glibenclamide in normal human volunteers

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**Abstract:** The study was aimed to evaluate various pharmacokinetic parameters of a commercially available fixed dose combination of oral antidiabetics (Metformin/Glibenclamide 500/5mg tablets) in plasma sample of normal healthy adult male volunteers by applying an accurate, selective, and reproducible HPLC-UV analytical method for quantification of Metformin HCL and Glibenclamide simultaneously in a single chromatographic run. Previously no HPLC-UV analytical method for simultaneous estimation of Metformin/Glibenclamide has been reported in Pakistan.

The human plasma samples were evaluated by using an isocratic High Performance Liquid Chromatography (HPLC) system of Sykam consisted of a pump with a column of Thermo Electron Corporation USA (ODS hypersil C<sub>18</sub> 4.6 mm x 250 mm), a UV-detector with data processing Clarity software. The mobile phase of 0.040M Potassium dihydrogen phosphate containing 0.25mL/L triethylamine at pH 3.5 (adjusted with 1:1 phosphoric acid) and acetonitrile (465:535v/v) was delivered with injection volume of 100µL at flow rate of 1 mL/min at 25°C temperature. The detection was performed at  $\lambda_{\text{max}}$  230 nm.

By applying this method, important pharmacokinetic parameters C<sub>max</sub>, T<sub>max</sub>, AUC<sub>0-∞</sub>, AUMC<sub>0-∞</sub>, t<sub>1/2</sub>, Ke, MRT, V<sub>d</sub> and Cl<sub>T</sub> are calculated. Maximum plasma concentrations C<sub>max</sub> was 131.856±8.050ng/ml for Glibenclamide (Mean ± SEM) and 511.106±12.675 ng/ml for Metformin HCL (Mean ±SEM).

**Keywords:** Metformin, glibenclamide, pharmacokinetic, plasma, HPLC.

## INTRODUCTION

Diabetes mellitus is a chronic disease and its management emphasis on maintaining blood glucose level as near to normal ("euglycemia") as approachable, without resulting in hypoglycemia.

To maintain normal blood glucose levels in patients having type-II diabetes mellitus who have not been stabilized on monotherapies, a combination of Metformin & Glibenclamide tablets were prepared with the aim to minimize the risk of long-term complications by decreasing the polypharmacy (Marre *et al.*, 2002; Flavia *et al.*, 2003). Combined formulations of metformin/ Glibenclamide have better glycemic control than the individual monotherapies in management of type-II diabetes and also facilitate the attainment of glycaemic control at lower doses of metformin or glibenclamide compared to respective monotherapies, without compromising tolerability (Marre *et al.*, 2002).

Therefore, this study was designed to evaluate various pharmacokinetic parameters of a commercially available combination brand in normal healthy adult male volunteers by applying a selective, accurate and reproducible HPLC method for quantification of

Metformin HCL and Glibenclamide simultaneously; both present in single oral tablet, in human plasma. This type of study has not been conducted yet in Pakistan, as confirmed by previously available literature.

While considering different chromatographic methods, High Performance Liquid Chromatography (HPLC) is proved to be more effective to attain separation, purification, identification and quantification of different compounds (Larson *et al.*, 2003). Implementation of various HPLC methods in the field of pharmaceutics and biomedicine has increased during the past decades and also for simultaneous estimation in biological fluids (Arayne *et al.*, 2010).

Previously, no analytical method has been reported and validated for simultaneous determination of Metformin HCL and Glibenclamide in human plasma samples using simple HPLC-UV analytical technique.

The present study was therefore, designed to evaluate various pharmacokinetic parameters of a commercially available combination brand of Metformin/Glibenclamide in normal healthy adult male volunteers by applying a selective, accurate and reproducible HPLC method for quantification of Metformin HCL and Glibenclamide simultaneously; both present in human plasma.

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## EXPERIMENTAL

### Materials

Metformin HCl and Glibenclamide were gifted by Pharmedic Laboratories Pvt. Ltd., Pakistan. Methanol HPLC grade was purchased from Merck, Germany. Distilled water was prepared locally in the Islamia University of Bahawalpur. Potassium dihydrogen phosphate, Phosphoric acid, Acetonitrile, Trichloroacetic acid and Triethylamine were purchased from Merck, Germany.

### Instruments

High Performance Liquid Chromatography (Sykam U.S.A.), Centrifuge Machine (Model 4000-China), pH Meter (WTW pH 300-Germany), Ultrasonic Bath (Fisher Scientific FS 28 H-Germany), Electric Balance (Percia XB 120A), Distillation Plant (WDA/4 R & M England), Ultra-low Freezer (Sanyo-Japan), Vortex Mixer (Seouline Bio-Sciince-Korea), Incubator (Velp Scientifica-Italy), Centrifuge machine (Hettich-Germany).

### Study Design

A randomized open, single dose cross over study design was adopted. Written informed consent was obtained from each subject before commencement of study. Eighteen healthy male human volunteers having age from 18-55 years and body weight in the range of 50-70/kg participated in the study. Each volunteer obtained oral single dose of metformin HCl 500mg + Glibenclamide 5/mg tablets, a fixed dose combination product (tablets) of Glucovance®-Merck.

### Sample Collection

A 20-gauge venous cannula was inserted into forearm for collection of blood samples. Blood samples were collected before administration of drug (zero time) and then at 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0 and 24.0 hours after dosing of Metformin HCl 500mg + Glibenclamide 5mg tablet. A 3ml blood sample was collected each time. Blood samples were centrifuged at 5000 rpm for 10 minutes and plasma was harvested. All samples were then frozen at -70°C in the ultra-low freezer (Sanyo-Japan, maximum -86°C) until assay. Stability of plasma samples were evaluated at -70°C for two months and at room temperature for 24 h. Under the above conditions, samples retained their potency (>95%).

### Preparation of the Mobile Phase

The mobile phase of 0.040 M Potassium dihydrogen phosphate containing 0.25mL/L triethylamine at pH 3.5 (adjusted with 1:1 phosphoric acid and acetonitrile, 465: 535v/v), was pumped at flow rate of 1 mL/min at ambient temperature. The injection volume was 100µL. The detection was performed at 230 nm.

### Preparation of Stock solutions

The stock solutions of MetGliben were prepared in triplicate by dissolving 100/mg in 100/mL of methanol

separately. Further dilutions were made from this stock solution in mobile phase. A combined standard solution in the range of 5.0 ng/mL to 625 ng/mL for Glibenclamide and 30 ng/mL to 625 ng/mL for Metformin HCl from two stock solutions were prepared.

### Preparation of Standard Curve

Standard curve was developed to encompass anticipated range of plasma concentrations of MetGliben found in healthy volunteers. Standard curve was prepared by spiking different samples of 1ml plasma to produce the calibration curve in range of 5.0-625 ng/mL for Glibenclamide and 30-625 ng/mL for Metformin HCl. Injections of 100/µl were injected and spectra were taken of each concentration. The peak areas were recorded for each concentration. The intra-day (within-run) and inter-day (between-run) accuracy and precision of method were determined on three separate days.

### Preparation of the sample (Extraction)

Combined standard solution (100/µl) was added to 1/mL of blank plasma (to prepare spiked plasma samples), vortexed for 5min., 1/ml of methanol was added and vortexed for 3min., trichloroacetic acid (10/µl) was added to precipitate plasma proteins, vortexed for 2 min and centrifuged (centrifuge, Hettich-Germany) at 5000 rpm for 10 min. The clear solution was transferred to polypropylene tube (1.5/mL) and 100/µl injected into HPLC system. Blank plasma samples (drugs free) were processed similarly as control samples.

### High Performance Liquid Chromatographic Conditions

An isocratic HPLC system of Sykam consisted of a pump with a column of Thermo Electron Corporation USA (ODS hypersil C<sub>18</sub> 4.6 mm x 250 mm), a UV- detector with data processing Clarity software employed to assay the prepared plasma samples. The UV detection of MetGliben was set at 230 nm. The mobile phase of 0.040 M Potassium dihydrogen phosphate containing 0.25mL/L triethylamine at pH 3.5 (adjusted with 1:1 phosphoric acid) and acetonitrile (465: 535v/v), was delivered at flow rate of 1 mL/min at ambient temperature.

### Pharmacokinetic Analysis

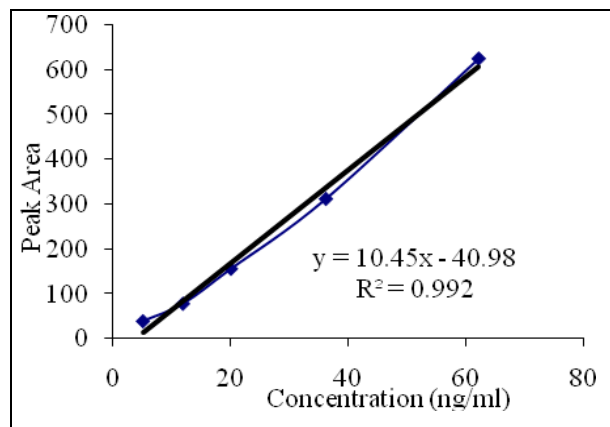
Pharmacokinetic parameters were calculated by non-compartmental pharmacokinetic method using Kinetica® PK/PD version 4.4.1 and Microsoft Excel 2007 for Windows 7. Maximum concentration of Metformin HCl and Glibenclamide in plasma (C<sub>max</sub>), time to reach peak plasma concentrations (T<sub>max</sub>) and other bioparameters (AUC<sub>0-∞</sub>, AUMC<sub>0-∞</sub>, t<sub>1/2</sub>, Ke, MRT, V<sub>d</sub> and Cl<sub>r</sub>) were determined by using above softwares.

## RESULTS

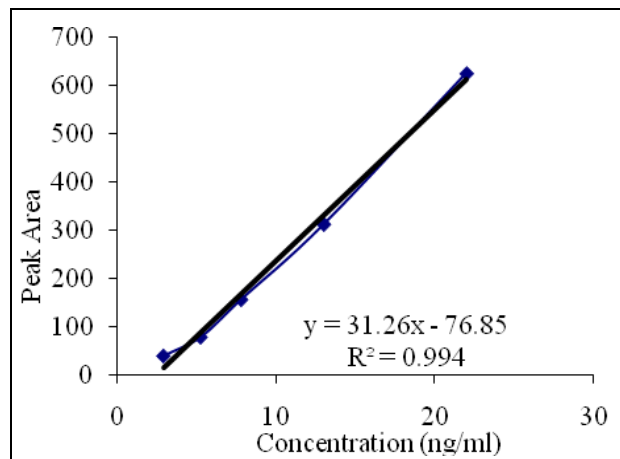
### Linearity

The standard curves of GLB (Glibenclamide) and MTF (Metformin) were prepared using known plasma concentrations within ranges of 5.0 ng/mL to 625 ng/mL

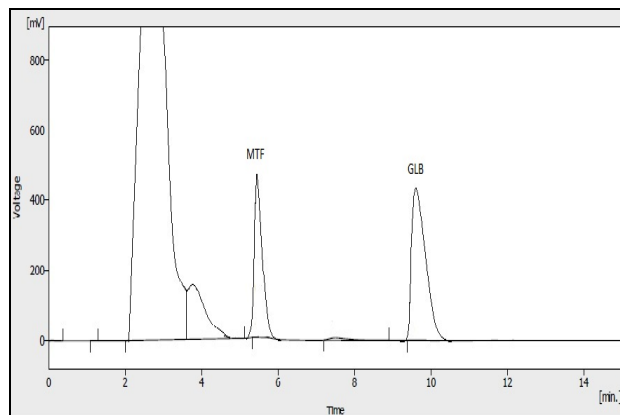
for GLB and 30 ng/mL to 625 ng/mL for MTF and Linear regression was applied to fit straight line. Mean  $r^2$  values for GLB and MTF were determined as 0.9930 and 0.9950, respectively and values of slope, intercept and  $r^2$  have shown in fig. 1 and 2. Retention times of MTF and GLB were  $5.5 \pm 1$  min and  $9.6 \pm 1$  min, respectively. A representative chromatogram has been shown in fig. 3.



**Fig. 1:** Standard Curve of Metformin HCL



**Fig. 2:** Standard Curve of Glibenclamide



**Fig. 3:** Spiked plasma with Metformin [MTF] and Glibenclamide (GLB).

### Precision and accuracy

Percent coefficient of variation (%CV) was calculated to find out intra-day and inter-day precision and accuracy of the present method for GLB and MTF in plasma. The findings are shown in table 1 and 2, respectively. The validation run was consisted of calibration curve and replicates ( $n=9$ ) of each low, medium and high quantification concentrations. For inter-day, analysis of three batches of each of the drug of GLB and MTF samples was performed on three different days.

**Table 1:** Intra-day and Inter-day precision and accuracy of GLB in human plasma ( $n=9$ )

Intra-day			
Glibenclamide			
Parameter	LQC (ng/mL)	MQC (ng/mL)	HQC (ng/mL)
Nominal Conc.	50.0	200	600
Mean	48.764	198.544	598.318
S.D.	0.2787	0.3876	6.845
Precision CV (%)	0.572	0.195	1.144
Accuracy (%)	97.53	99.27	99.72
Inter-day			
Glibenclamide			
Parameter	LQC (ng/mL)	MQC (ng/mL)	HQC (ng/mL)
Nominal Conc.	50.0	200	600
Mean	47.998	197.522	596.668
S.D.	0.616	0.7542	9.127
Precision CV(%)	1.283	0.382	1.529
Accuracy (%)	95.99	98.76	99.44

**Table 2:** Intra-day and Inter-day precision and accuracy of MTF in human plasma ( $n=9$ )

Intra-day			
Metformin			
Parameter	LQC (ng/mL)	MQC (ng/mL)	HQC (ng/mL)
Nominal Conc.	50.0	200	600
Mean	49.665	197.116	599.02
S.D.	0.406	1.2776	8.223
Precision CV (%)	0.817	0.648	1.373
Accuracy (%)	99.33	98.56	99.83
Inter-day			
Metformin			
Parameter	LQC (ng/mL)	MQC (ng/mL)	HQC (ng/mL)
Nominal Conc.	50.0	200	600
Mean	49.487	196.879	598.153
S.D.	0.8612	2.2117	9.768
Precision CV (%)	1.740	1.123	1.633
Accuracy (%)	98.97	98.44	99.69

### Quantification Limits

Limit of detection (LOD) and Limit of quantitation

(LOQ) of GLB and MTF as mean $\pm$ SD were 2.0 $\pm$ 0.376ng/mL & 4.0 $\pm$ 0.227ng/mL and 28.0 $\pm$ 0.528ng/mL & 30.0 $\pm$ 0.466ng/mL for GLB and MTF, respectively.

Lower quantitation limits showed the higher sensitivity of present method for these drugs in single run.

#### Extraction yields

Percent extraction yield was calculated by comparing mean drug concentration from spiked plasma samples (extracted) with mean concentration from standard solutions (true solutions of GLB and MTF), determined from response (peak areas). Mean extraction recoveries were determined by analyzing four replicates of plasma samples at three concentration levels of each drug of GLB and MTF combination. The values of extraction yields are presented in table 3 for both drugs.

#### Ruggedness

The ruggedness of the HPLC method was evaluated by carrying out the analysis using standard solution, same chromatographic system and column on different days. Small differences in response were observed with RSD% as 0.34. Similarly, ruggedness was also checked by

injecting the standard solution in different HPLC systems (Agilent series 1200 and Perkin Elmer series 200). The developed method was found satisfactorily rugged which is indicated by high degree of detector responses and retention times.

#### Freeze and Thaw stability

The stability of GLB and MTF in human plasma was assessed by analyzing replicates (n = 9) of low, medium and high dilution of plasma samples during the sample storage and processing procedures. All samples were processed in dark to protect from photo-oxidation and stored at -20°C in ultra-low freezer (Sanyo, Japan). From zero to three freeze-thaw cycles (cycle 0, cycle 1, cycle 2 and cycle 3), stability studies were performed and have been illustrated in table 4.

#### Plasma concentration profile

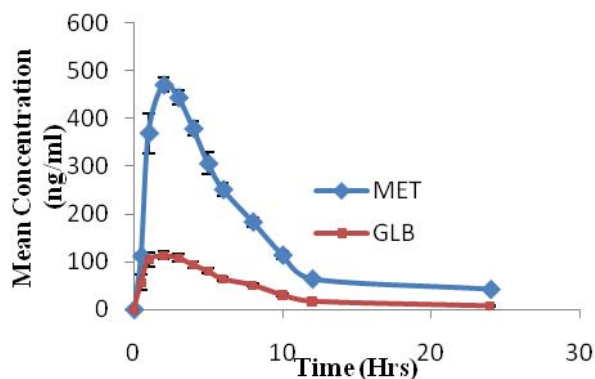
The individual plasma concentration profiles of Metformin HCl/Glibenclamide 500/5mg were determined in 18 volunteers at given time schedule. Fig. 4 is representing mean plasma concentration profile of Metformin/Glibenclamide in 18 volunteers.

**Table 3:** Percent extraction yield of GLB and MTF in human plasma (n=9)

Glibenclamide				
Parameters	Conc. Added (50 ng/ml)		Conc. Added (100 ng/ml)	
	Conc. Found in spiked samples	Conc. Found in standard solutions	Conc. Found in spiked samples	Conc. Found in standard solutions
Mean $\pm$ S.D.	48.185 $\pm$ 0.3728	49.072 $\pm$ 0.2381	98.240 $\pm$ 1.566	99.673 $\pm$ 0.997
CV (%)	0.7737	0.4852	1.594	1.00
PEY (%)	98.19		98.56	
Metformin				
Parameters	Conc. Added (50 ng/ml)		Conc. Added (200 ng/ml)	
	Conc. Found in spiked samples	Conc. Found in standard solutions	Conc. Found in spiked samples	Conc. Found in standard solutions
Mean $\pm$ S.D.	49.137 $\pm$ 0.4118	49.879 $\pm$ 0.3871	198.213 $\pm$ 1.819	199.694 $\pm$ 1.298
CV (%)	0.8381	0.7761	0.9177	0.6499
PEY (%)	98.51		99.26	

**Table 4:** Freeze thaw stability in plasma for GLB and MTF (n=9)

Glibenclamide								
Parameters	Freeze-Thaw Cycles							
	Cycle 0 ng/ml		Cycle 1 ng/ml		Cycle 2 ng/ml		Cycle 3 ng/ml	
Nominal conc.	50.0	100	50.0	100	50.0	100	50.0	100
Mean $\pm$ SD	48.659 $\pm$ 0.2816	98.719 $\pm$ 1.239	48.127 $\pm$ 0.4181	98.417 $\pm$ 0.937	47.989 $\pm$ 0.4782	98.113 $\pm$ 0.828	47.733 $\pm$ 0.5153	97.878 $\pm$ 0.866
CV (%)	0.5787	1.255	0.8687	0.9521	0.9969	0.8439	1.0796	0.8848
Difference	-	-	-0.532	-0.302	-0.670	-0.606	-0.926	-0.841
Metformin								
Nominal conc.	50.0	200	50.0	200	50.0	200	50.0	200
Mean $\pm$ SD	49.7141 $\pm$ 0.4805	198.610 $\pm$ 0.919	49.281 $\pm$ 0.4973	198.157 $\pm$ 1.218	48.918 $\pm$ 0.5661	197.683 $\pm$ 1.714	48.636 $\pm$ 0.7112	196.888 $\pm$ 1.099
CV (%)	0.9665	0.4627	0.4331	0.6147	1.1572	0.8670	1.4623	0.5582
Difference	-	-	-0.4331	-0.453	-0.7961	-0.927	-1.0781	-1.722



**Fig. 4:** Mean (n=18) plasma concentration vs. time profile of GLB and MTF 5 mg and 500 mg plotted on rectangular co-ordinate graph.

## DISCUSSION

The prime focus of current study was to assess the pharmacokinetic parameters of two combined drug moieties Glibenclamide and Metformin in a single tablet dosage form.

The maximum plasma drug concentration ( $C_{max}$ ) represents maximum plasma drug concentration obtained after oral administration of drug. For many drugs, a relationship is found between pharmacological and therapeutic effects of the drugs and plasma concentration. The pharmacological response is however, directly proportional to the plasma concentration of drugs.  $C_{max}$  is an indicator of rate of absorption of drugs i.e. higher the peak concentration, faster the rate of absorption.  $C_{max}$  gives an indication that the drug is sufficiently systemically absorbed to provide therapeutic response. In addition,  $C_{max}$  also give warning of possible toxic levels of drugs (Shargel and Yu, 1999).

In this study, maximum plasma concentrations (Mean  $\pm$  SEM) of Glibenclamide found to be  $131.856 \pm 8.050$  ng/ml. In a previous study, conducted according to same study design (using Glibenclamide as the only active drug moiety),  $C_{max}$  was  $167.7 \pm 63.4$  ng/ml (Ioannis and Athanasios, 2002) while other study using MS detection has reported  $C_{max}$  as  $203.8 \pm 53.4$  ng/ml (Florin *et al.*, 2007). Here the  $C_{max}$  is not comparable with the previous studies. According to Niemi and Cascorbi, 2002, Glibenclamide is metabolized by Cytochrome P450 (CYP) 2C9 which is polymorphically expressed. Pharmacokinetic variation of drug in individuals is possible because of enzymatic differences. Variation in  $C_{max}$  was observed within the therapeutic safety index of drugs that does not affect the therapeutic effects of drug. Maximum plasma concentrations,  $C_{max}$  (Mean  $\pm$  SEM) of Metformin in the present study found to be  $511.106 \pm 12.675$  ng/ml. While another study conducted by Bhaveshin 2007, reported  $C_{max}$  of Metformin as  $769.0368$

$\pm 177.75$  ng/ml. Here the difference in  $C_{max}$  may be due to the difference in dose strength i.e. current study utilized 500mg while in previous study the dose administered was 850mg.

At  $T_{max}$  maximum drug absorption occurs and the rate of drug absorption exactly equals to the rate of drug elimination (Shargel and Yu, 1999). In the present study,  $T_{max}$  values (Mean  $\pm$  SEM) of Glibenclamide was  $1.889 \pm 0.196$  hours. According to a previous research conducted on glibenclamide as an individual drug,  $T_{max}$  values (Mean  $\pm$  SEM) were 3.6 hours and 2.6 hours (Ioannis and Athanasios, 2002; Florin *et al.*, 2007). So this pharmacokinetic parameter of simultaneous study is not comparable with the previous one. This difference in  $T_{max}$  may be due to two reasons. One may be that the reported study has employed female volunteers as well and that study has used micronized tablets of glibenclamide. While in current study all the volunteers were male and dosage form was immediate release tablet. The Food and Drug Administration (FDA) studied various new drug applications and in some drugs observed 40% pharmacokinetic variability due gender difference. Among one of these studies, females had significantly longer gastric residence time (GRT) that ultimately delayed the absorption of drugs when administered with meal as compared to males (Gail, 2005). This delay in GRT leads towards greater values of  $T_{max}$  in females. From this very fact one can conclude that the difference in the  $T_{max}$  of two studies may be due to gender difference.

$T_{max}$  value (Mean  $\pm$  SEM) of Metformin was  $2.056 \pm 0.151$  hours. According to a previously conducted simultaneous study,  $T_{max}$  of metformin was 2.6 hours (Christina *et al.*, 2007) which is comparable with the present study.

The extent of absorption signifies fraction of the administered dose that is actually absorbed and appears in the blood stream. Since, it is not possible to determine directly the fraction of administered dose that is actually absorbed. The area under the plasma concentration vs time curve (AUC) is used as an indicator to estimate the extent of absorption (Madan, 2000). AUC is the total amount of active drug that reaches the systemic circulation (Shargel and Yu, 1999). In the present study; the value of  $AUC_{0-\infty}$  (Mean  $\pm$  SEM) of Glibenclamide was  $927.713 \pm 57.483$  ng. h/ml. The value of  $AUC_{0-\infty}$  was reported as  $878.3 \pm 306.5$  by Ioannis and Athanasios (2002) while in another study, it was found as  $953 \pm 52.6$  ng/ml (Florin *et al.*, 2007). This shows that in case of simultaneous evaluation of glibenclamide with metformin, it possesses almost same extent of absorption. The value of  $AUC_{0-\infty}$  (Mean  $\pm$  SEM) of Metformin was  $3606.427 \pm 82.732$  ng. h/ml while in a previous study it was  $5721.8925 \pm 1189.5$  ng. h/ml (Bhavesh *et al.*, 2007). The differences in administered dose in both studies (i.e. 500mg versus 850mg) may alter the values of AUC.

Half-life of a drug represents the time necessary for the concentration of unbound drug in the blood reduced to one half from its initial value. The plasma half life ( $t_{1/2}$ ) of Glibenclamide was  $5.251 \pm 0.198$  hours (Mean  $\pm$  SEM). Previously reported half life of a single drug study was 3.4 hours (Ioannis and Athanasios, 2002) and in another study it was 7.9 hours (Florin *et al.*, 2007). In present study, the plasma half-life ( $t_{1/2}$ ) of Metformin was 5.738 hours which is in good agreement with previously reported value of half life i.e. 4.0 hours (Scheen, 1996).

Distribution of Metformin is fast after its absorption and does not bind to plasma proteins. No metabolites or conjugates of metformin have previously been reported. The absence of liver metabolism clearly differentiates the pharmacokinetics of metformin from that of other biguanides, such as phenformin. Metformin undergoes renal excretion and has a mean plasma elimination half-life between 4.0 and 8.7 hours. Renal disorders can prolong the elimination of metformin and correlates with creatinine clearance (Scheen, 1996).

The volume of distribution more appropriately known as the "apparent volume of distribution" is the volume of fluid in which the drug appears to be uniformly distributed. The values of volume of distribution ( $V_d$ ) of Glibenclamide and Metformin (Mean  $\pm$  SEM) was  $40.903 \pm 2.527$  L and  $1087.397 \pm 32.841$  L, respectively. Glibenclamide exhibit extensive plasma protein binding (99%) (Jonsson *et al.*, 1998) which may be responsible for less volume of distribution of Glibenclamide as compared to oral hypoglycemic agents of biguanides class (i.e. Phenformin or Metformin etc.). Metformin represents high apparent volume of distribution (300-1000 L after a single dose) as it shows negligible plasma protein binding (Young *et al.*, 2006).

Clearance is one of the parameter that determines the maintenance dose rate required to achieve a target plasma concentration and therefore, effect at steady state.

The total body clearance ( $Cl_T$ ) (Mean  $\pm$  SEM) value of Glibenclamide was  $5.465 \pm 0.340$  L/h. It is suggested for drugs like glibenclamide which extensively bound to plasma proteins have very small changes in the apparent volume of distribution (Morrison *et al.*, 1982). As in the current study, clearance is low, and therefore, it is likely to be a function of plasma protein binding.

The total body clearance ( $Cl_T$ ) (Mean  $\pm$  SEM) values of Metformin was  $131.172 \pm 2.848$  L/h. Renal clearance of metformin is approximately 3.5 times greater than creatinine clearance, which indicates that tubular secretion is the major route of metformin elimination. Following oral administration, approximately 90% of the absorbed drug is eliminated via renal route within first 24 hours, with a plasma elimination half-life of

approximately 6.2 hours (Barbieri and Robert, 2003). As the major portion of absorbed drug is eliminated via renal route by larger active tubular secretion that is why the value of total body clearance of metformin in current study is high,

## CONCLUSION

For the simultaneous assay of metformin and glibenclamide in human plasma samples, an analytical method is developed. The method is suitable for the pharmacokinetic studies due to its sensitivity, time and cost effectiveness.

As the method is accurate and sensitive, it is successfully used for the assessment of various pharmacokinetic parameters (like  $C_{max}$ ,  $T_{max}$ ,  $AUC_{0-\infty}$ ,  $AUMC_{0-\infty}$ ,  $t_{1/2}$ ,  $K_e$ ,  $MRT$ ,  $V_d$  and  $Cl_T$  etc.) of commercially available pharmaceutical formulations (immediate release tablets) containing 500/mg metformin and 5/mg glibenclamide per dose. Thus, this method can be useful in real clinical situations. Moreover, the proposed method is reliable, thus can also be used for the conduct of bioequivalence studies.

The presented pharmacokinetic parameters indicate that pharmacokinetic profile of combination therapy is same as that of individual drug therapy.

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