5.0 DISCUSSION

5.1 FIXED DOSE COMBINATION

The development of FDCs is becoming increasingly important from a public health perspective. FDCs have advantages when there is an identifiable patient population for whom treatment with a particular combination of active substances in a fixed ratio of doses has been shown to be safe and effective and when all of the active substances contribute to the overall therapeutic effect. Medical experts world over have been expressing serious concerns over the marketing of fixed dose combination by pharmaceutical companies. Each fixed dose combination should be carefully justified and clinically relevant (e.g. in cases when each component of the FDC has several possible dosages and dosages that have shown benefit on clinical outcomes may be preferable).

FDCs have potentially lower costs of manufacturing compared with the costs of producing separate products administered concurrently, simpler logistics of distribution and improved patient adherence (WHO 2005). They are being used in the treatment of a wide range of conditions and are particularly useful in the management of chronic conditions like diabetes and dyslipidemia.

Diabetes has become an increasingly prevalent and costly chronic disease state and presents a significant medical and economic burden to health care system (Mokdad et al. 2000). Much of the morbidity and cost is attributable to long term diabetes-related complications, particularly cardiovascular disease (CVD) (Mark et al. 2008). Diabetes is a group of syndromes that leads to various other complications that increase morbidity and mortality (Cerveny et al. 1988). For example, patients with diabetes are at risk for microvascular complications such as retinopathy, nephropathy and neuropathy, moreover for macrovascular complications such as myocardial infarction.

Prevention of these complications requires a management strategy that addresses multiple physiological conditions including hyperglycemia, dyslipidemia and hypertension (Mark et al. 2008). Therefore, use of an FDC is an appropriate strategy for improving medication adherence in diabetic patients who require multiple drug therapy.
It is well established that type-2 diabetes is associated with a 2 to 4 fold excess risk of coronary heart disease (Haffner 1998). Patients with type-2 diabetes typically have a preponderance of smaller and denser LDL cholesterol particles, which may increase atherogenicity even if the absolute concentration of LDL cholesterol is not significantly increased (Haffner 1998). In SHIELD (Study to Help Improve Early evaluation and management of risk factors Leading to Diabetes) a multivariate analysis of self-reported data from 22,001 patients showed that dyslipidemia was independently associated with a higher likelihood of type-2 diabetes diagnosis (odds ratio, 3.95, P <0.0001) (Bays et al. 2007).

Data from CARDS (Collaborative Atorvastatin Diabetes Study) demonstrated a clinical benefit (37% reduction in the first incidence of a major cardiovascular event, P = 0.001) with Atorvastatin in patients (n = 2838) with type-2 diabetes whose lipid profile was not highly elevated, highlighting the importance of lipid modification for primary prevention of CVD in patients with type-2 diabetes (Colhoun et al. 2004). Baseline data from the UKPDS (UK Prospective Diabetes Study) showed that both decreased high-density lipoprotein (HDL) cholesterol and elevated LDL cholesterol predict CHD (Turner et al. 1998).

In the 4S (Scandinavian Simvastatin Survival Study) trial, simvastatin significantly reduced CHD incidence and total mortality in diabetic subjects with high LDL cholesterol and previous clinical CHD (Herman et al. 1999). In the CARE (Cholesterol And Recurrent Events) study, pravastatin reduced CHD incidence significantly in diabetic subjects with mean LDL cholesterol levels of 139 mg/dL and previous clinical CHD (Plehn et al. 1999).

Of all available cholesterol-lowering treatments, statins are the most effective at decreasing the risk of total stroke (De et al. 2010). Studies have shown that high-dose statin therapy is effective in achieving LDL-C goals and associated with favorable effects on lipoprotein subfractions in patients with type-2 diabetes, which may translate into clinical benefits in terms of anti-atherogenic potential and a subsequent reduction in the risk of adverse cardiovascular outcomes (Lawrence et al. 2004). Among the statins, Atorvastatin 10 mg/day is the most cost effective cholesterol lowering drug (Plans et al. 2006). A 4-year follow-up study showed that the use of Atorvastatin at a dose of 10 mg/day in type-2 diabetes was able to reduce the risk of
cardiovascular events by 35% (Colhoun et al. 2004). The greater efficacy of Atorvastatin than other currently available statins is believed to be due to a prolonged duration of HMG-CoA reductase inhibition (Malhotra et al. 2001).

Hence, Atorvastatin was chosen for the FDC in combination with Metformin for a population of diabetic patients with a risk of CHD/dyslipidemia. Metformin is considered a drug of first choice for diabetes. Its disposition is apparently unaffected by the presence of diabetes and only slightly affected by the use of different oral formulations (Scheen et al. 1996). Metformin is rapidly distributed following absorption, does not bind to plasma proteins and is not metabolized in the liver (Scheen et al. 1996). UKPDS also showed that Metformin was not associated with weight gain (UKPDS-34, Bolen et al. 2007).

No metabolites or conjugates of Metformin have been identified. There is no significant interaction between Atorvastatin and Metformin (Scheen et al. 2005). In addition to their glucose-lowering properties, antidiabetic agents that directly improve insulin resistance may have effects on lipid levels and especially TG levels. Although there may be no effect on HDL-C levels, these agents may instead alter the ratio of lipoproteins in HDL towards more anti-atherogenic HDL particles (Moon and Kashyap 2004).

Metformin has been shown to reduce LDL-C, TC and TG levels, but increase HDL-C levels (Glucophage 2009). Similarly, pioglitazone has been shown to reduce TG levels and increase HDL-C levels (Actos 2009). In contrast, rosiglitazone has been shown to increase LDL-C, TC and HDL-C levels, although this thiazolidinedione does not affect TG levels (Avandia 2008). Additionally, the efficacy, safety and tolerability of an FDC of Atorvastatin/Metformin sustained-release (SR) 10mg/500mg were assessed in a pharmacodynamic study conducted in adult Indian patients with diabetic dyslipidemia (Balasubramanian et al. 2008).

Therapy with this FDC resulted in a significant reduction in mean plasma fasting and postprandial glucose levels. There was a steep fall in glycosylated haemoglobin (HbA1c) level, a significant reduction in mean total cholesterol, LDL cholesterol and very LDL cholesterol levels, moreover a significant increase in mean HDL cholesterol level was reported. The results of this study suggested that an FDC of Atorvastatin/Metformin SR 10mg/500mg is an efficacious and well tolerated
therapeutic modality in patients with diabetic dyslipidaemia (Balasubramanian et al. 2008). The effect of combined therapy with Atorvastatin and Metformin on glucose-induced variations in inflammatory molecules was studied in patients with newly diagnosed type-2 diabetes (Tousoulis et al. 2011). Serum tumor necrosis factor (TNF-α) level was reduced significantly after treatment with Metformin 850 mg/day plus Atorvastatin 10 mg/day compared with Metformin alone. The results of this study also showed that the combination of Metformin and Atorvastatin partly prevented glucose-loading-induced elevation of postprandial glucose levels (at 1 hr), suggesting a better response to glucose intake than monotherapy with Metformin (Tousoulis et al. 2011). Therefore, the results of published studies clearly demonstrate clinical benefits for an FDC of Atorvastatin and Metformin in diabetic patients. Based on these facts, the Atorvastatin/Metformin ER 10mg/500mg FDC tablet was developed and bioequivalence was evaluated in healthy subjects against co-administration of individual formulations of Atorvastatin and Metformin in corresponding doses.

5.2 CLINICAL EVALUATION

The clinical study was carried out in accordance with ICH Good Clinical Practices. The study protocol and the informed consent form were approved by the Jamia Hamdard Institutional Review Board. This study was designed based on the known pharmacokinetics of the study drug, Metformin and Atorvastatin on the generally accepted standards for the conduct of bioequivalence studies.

Each of the subjects was required to read and understand the information before giving his consent to participate in the study by signing the informed consent form. The signed original copy was retained and one signed copy was given to the study subject for the record. They were instructed during screening not to take any prescription and OTC medications until the completion of the study.

The study formulations (test T and reference R) were planned to be evaluated in 40 subjects. Thirty four subjects were administered both test and reference formulations. Subject number 07 was not dosed with any of the drug formulation. Subject numbers 05, 08 and 24 were dosed only with test formulation in Period I. Subject numbers 16 and 25 were dosed only with reference formulation in Period I.
The clinical examination of the subjects was performed by a physician. Clinical examination with medical history was performed at screening and brief clinical examination after admission and at discharge in each period. Only subjects who had clinically normal laboratory profiles as well as fulfilled the inclusion and exclusion criteria were enrolled in the study. The clinical examination of all the subjects was found to be normal in both periods of the study.

A single oral dose of fixed dose combination tablet of Metformin HCl and Atorvastatin calcium 500+10 mg of either test or Lipitor® tablet 10 mg and Glucophage® XR 500 mg tablet administered concurrently of reference formulation was administered during each period of the study. The order of receiving the test and reference products for each subject was determined according to a SAS generated randomization schedule. During the study, the assigned doses were administered under the supervision of trained study personnel. The oral cavity was checked after dosing to ensure compliance with the treatment.

In the interest of subject’s safety and acceptable standards of medical care the investigators or medical officers were permitted to prescribe treatment(s) at their discretion, the details of which were recorded in concomitant medication form.

Adverse event monitoring was done throughout the study. Eleven adverse events were observed during the study, amongst them four was observed from test treatment groups and seven adverse events were observed from reference treatment groups. Concomitant medication was prescribed for some of the adverse event reported during the study. There were no serious and significant adverse events or deaths during the conduct of the study.

The test and reference product were well tolerated by the study subjects in the study.

5.3 ANALYTICAL EVALUATION

Bioanalytical method validation (BMV) employed for the quantitative determination of drugs and their metabolites in biological fluids plays a significant role in the evaluation and interpretation of bioavailability, bioequivalence, pharmacokinetic and toxicokinetic study data. The quality of these studies is directly related to the quality of the underlying bioanalytical data. A validated bioanalytical method is required for
the regulatory acceptance of bioequivalence and pharmacokinetic data (Bioanalytical MV guidelines by FDA, 2001). Bioanalytical functions in the pharmaceutical industry are constantly under pressure to reduce the development time, this is often accompanied with an increase in the number of biological samples requiring pharmacokinetic analysis and decrease in the desired quantitation level.

Liquid chromatography-mass spectrometry (LC-MS/MS) is an analytical technique that has led to major breakthrough in the field of quantitative bioanalysis, due to its inherent specificity, sensitivity and speed. A number of high-performance liquid chromatography methods are available with ultra-violet detection (Altuntas et al. 2004, Bahrami et al. 2005, Zarghi et al. 2005; Shen et al. 2006), enzyme inhibition (Shum et al. 1998; Valesky et al. 2008) and liquid chromatography-tandem mass spectrometry (Hermann et al. 2005, Borek-Dohalsky et al. 2006, Nirogi et al. 2006, Ma et al. 2007, Liu et al. 2008, Guillen et al. 2009; Novakova et al. 2009) for the estimation of Atorvastatin in human plasma in literature. High-performance liquid chromatography with ultra-violet or electrochemical detection methods typically has a higher limit of quantification and is more time consuming. Undeniably, mass spectrometry has become the method of choice for quantification of metabolite concentration in biological matrices due to its superior selectivity.

For Atorvastatin a number of HPLC (high performance liquid chromatography) methods have been published, by using various separation modes, such as (Jemal et al. 1999; Bullen et al. 1999) liquid–liquid extraction (LLE). Sample preparation by solid phase extraction (SPE) was desirable in order to reduce the amount of organic solvent used, get cleaner extracts, higher recoveries and make the process easy.

For the estimation of Metformin, a number of HPLC (high performance liquid chromatography) methods have been published by using various separation modes, such as reverse phase (Tache et al. 2001), ion-pair (AbuRuz et al. 2003, David et al. 2005; Ranetti et al. 2009), cation exchange (Imre et al. 2006) and normal phase chromatography (Cheng et al. 2001) in human plasma. Several methods using liquid-chromatography tandem mass spectrometry (LC/MS/MS) (Heinig et al. 2004, Chen et al. 2004, Wang et al. 2004; Koseki et al. 2005) are also available for the analysis of Metformin in plasma. Some involve protein precipitation with an organic solvent, without an evaporation step (Chen et al. 2004; Heinig et al. 2004) and others use
protein precipitation combined with liquid-liquid extraction (Wang et al. 2004) or cation exchange solid-phase extraction (Koseki et al. 2005).

The HPLC methods have disadvantages of longer run times, lack of sensitivity, use of complex sample preparation procedures (Tache et al. 2001) and use of large sample volumes (AbuRuz et al. 2003; Tache et al. 2001). LC/MS/MS methods have the advantage of a higher sensibility, higher selectivity and higher throughput (Cristina et al. 2010). LC-MS/MS as an instrument has become the method of choice. Most of the people have used precipitation as the sample processing technique while using mass spectrometry.

Based on the current regulatory requirements, a liquid chromatography mass spectrometry method for the estimation of Atorvastatin in human plasma was developed. Keeping in mind the number of plasma samples from a BA/BE study, solid phase extraction (SPE) was used as the sample processing technique. The advantage with SPE is to reduce the amount of organic solvent used, get cleaner extracts, higher recoveries and make the process easy. For the estimation of Metformin in human plasma a liquid chromatography mass spectrometry method was developed and protein precipitation was used as the sample processing technique.

The method was validated in terms of selectivity, precision, accuracy, linearity, recovery, dilution integrity, ruggedness and stability studies. Stability studies includes freeze-thaw, bench top, in-injector, long term and stock solution. Before the initiation of unknown plasma samples of a biostudy, validation was completed and all validation parameters met the acceptance criteria. The acceptance criteria of international regulatory standards were followed.

For Atorvastatin, o-Hydroxy Atorvastatin, p-Hydroxy Atorvastatin, selectivity using twelve lots of plasma were evaluated and none shows interferences at drug and internal standard retention times. Method was found to be precise and accurate. This was confirmed by three precision and accuracy batches. For Atorvastatin, p-Hydroxy Atorvastatin and o-Hydroxy Atorvastatin the within batch precision was less than 5.3%, 9.3% and 6.0% respectively and between batch precision was less than 7.8%, 7.3% and 7.1% respectively. For Atorvastatin, p-Hydroxy Atorvastatin and o-Hydroxy Atorvastatin, within batch accuracy ranged from 92.3% to 106.9%, 96.5% to 111.7% and 86.1% to 103.1% respectively and between batch accuracy for
Atorvastatin, p-Hydroxy Atorvastatin and o-Hydroxy Atorvastatin ranged from 95.6\% to 102.3\%, 97.4\% to 110.4\% and 91.0\% to 102.1\% respectively. Ruggedness of the method was also performed and precision/accuracy was within the acceptable limits. Matrix effect evaluation was performed on six lots of plasma batches and matrix effect was not observed with the method. For Atorvastatin, p-Hydroxy Atorvastatin and o-Hydroxy Atorvastatin recovery was 87.34\%, 92.48\% and 85.75\% respectively and was found consistent between low, middle and high concentration. Atorvastatin, p-Hydroxy Atorvastatin and o-Hydroxy Atorvastatin were found stable during three freeze thaw cycles. Bench top stability was performed for 7.52 hr and Atorvastatin, p-Hydroxy Atorvastatin and o-Hydroxy Atorvastatin was found stable for that period. In reconstituted samples, when kept in auto-sampler, Atorvastatin, p-Hydroxy Atorvastatin and o-Hydroxy Atorvastatin was found quiet stable for 50.38 hr. Stability was also confirmed when plasma containing Atorvastatin, p-Hydroxy Atorvastatin and o-Hydroxy Atorvastatin was kept for 90 days below -50 °C, protected from light. Stock solution stability was conducted at 2-10 °C (refrigerated temperature) for Atorvastatin, p-Hydroxy Atorvastatin and o-Hydroxy Atorvastatin and their respective internal standards and the results passed the acceptance criteria.

For Metformin, selectivity was evaluated using twelve lots of plasma and none shows interferences at drug and internal standard retention times. Method was found to be precise and accurate. This was confirmed by four precision and accuracy batches. For Metformin the within batch precision was less than 6.4\% and between batch precision was less than 7.7\%. For Metformin within batch accuracy ranged from 87.9\% to 105.8\% and between batch accuracy ranged from 96.8\% to 100.2\%. Ruggedness of the method was also performed and precision/accuracy was within the acceptable limits. Matrix effect evaluation was performed on six lots of plasma batches and matrix effect was not observed with the method. For Metformin recovery was 84.03\% and was found consistent between low, middle and high concentration. Metformin was found stable during three freeze thaw cycles. Bench top stability was performed for 15.62 hr and Metformin was found stable for that period. In reconstituted samples, when kept in auto-sampler, Metformin was found quiet stable for 43.80 hr. Stability was also confirmed when plasma containing Metformin was kept for 50 days below -50 °C, protected from light. Stock solution stability was conducted at 2-10 °C
(refrigerated temperature) for Metformin and their internal standard and the results passed the acceptance criteria.

During actual conduct of the study, all the above mentioned parameters were kept in mind. Subject samples did not exceed three freeze thaw cycles. Processing of each subject took 3-4 hr which is well within our bench top stability data. For Atorvastatin, p-Hydroxy Atorvastatin and o-Hydroxy Atorvastatin auto-sampler time did not exceed 50.38 hr and for Metformin auto-sampler time did not exceed 43.8 hr during subject analysis. No concomitant medication was used for all completed subjects. Samples from single subject were run against one calibration curve along with two sets of quality control samples (LQC, M1QC, MQC and HQC) interspersed between subject samples. System suitability was run on each day before initiation of run. \( C_{\text{max}} \) of Atorvastatin and Metformin was found matching with the literature, which confirms the accuracy of the method.

### 5.4 PHARMACOKINETIC EVALUATION

The current bioequivalence standards to establish bioequivalence of generic drug products typically compare blood levels of drug for standard and reference products over time after a single dose to volunteer subjects. From these data, the maximum observed blood concentration \( (C_{\text{max}}) \) and the area under the blood level versus time curve \( (\text{AUC}) \) are calculated for each product using a logarithmic transformation.

In this study the plasma concentration versus time profiles obtained for Atorvastatin and Metformin after administration of the FDC and co-administration of Atorvastatin 10 mg and Metformin 500 mg ER tablets to healthy subjects in two different periods was very similar and in fact essentially super imposable.

The power obtained in the study for \( C_{\text{max}} \) and AUC was more than 98% for both Atorvastatin and Metformin. The 90% CIs obtained for Atorvastatin for \( C_{\text{max}} \), \( \text{AUC}_{0-\infty} \) were \( (88.11-106.93), (91.18-107.94) \) and \( (89.25-106.60) \) respectively. The 90% CIs obtained for Metformin for \( C_{\text{max}} \), \( \text{AUC}_{0-\infty} \), \( \text{AUC}_{0-24} \) and \( \text{AUC}_{0-\infty} \) were \( (113.3-124.0), (102.65-117.97), (101.87-116.82) \) and \( (102.44-117.53) \) respectively.

The pharmacokinetic results of the study clearly showed that the two treatments yielded similar pharmacokinetic profiles for Atorvastatin and Metformin in healthy subjects. Both treatments were well tolerated by the subjects. The 90% CIs for
pharmacokinetic parameters ($C_{\text{max}}$ and AUC) for Atorvastatin and Metformin were well within regulatory acceptance criteria and demonstrating bioequivalence between the two treatments under fed conditions.

The results obtained in this study indicate that the Atorvastatin/Metformin ER 10mg/500mg FDC can be safely administered to diabetic patients with a risk of CHD and will reduce their pill burden. Data from the literature also show that the combination of Atorvastatin and Metformin is safe (Tousoulis et al. 2011). This FDC can increase patient compliance with treatment and hence also improve the quality of life of diabetic patients.

Statins are an important component of the medical management of patients with diabetes. Atorvastatin is well tolerated in patient populations. An analysis was performed to evaluate in detail the safety and tolerability profile of Atorvastatin 10 mg compared with placebo among 2838 patients in the CARDS (Collaborative Atorvastatin Diabetes Study) safety population (Newman et al. 2008).

The analysis showed that the incidence of adverse events observed in the Atorvastatin and placebo treated groups was comparable. The analysis also demonstrated the safety of Atorvastatin 10 mg over a median follow-up period of 3.9 years in patients with type-2 diabetes with and without elevated LDL cholesterol (Newman et al. 2008). Metformin is the only biguanide available in most countries. It is generally well tolerated, with the most common adverse effects being gastrointestinal (Nathan et al. 2006). Although always a matter of concern because of its potentially fatal outcome, lactic acidosis is a relatively rare (<1 case per 100 000 treated patients) adverse effect. Metformin monotherapy is usually not accompanied by hypoglycaemia and has been used safely in patients (Nathan et al. 2006).

In response to safety concerns about Metformin, Bristol-Myers Squibb commissioned a large study (COSMIC; The Comparative Outcomes Study of Metformin Intervention versus Conventional Approach) comparing 1 year of treatment with Metformin with ‘usual care’ with other antihyperglycaemic agents (Khurana et al. 2010). The results showed no differences in safety outcomes between the 7227 patients who received Metformin and the 1505 patients who received usual care. There were no cases of lactic acidosis in either group (Khurana et al. 2010).
Furthermore, taking Metformin may be associated with a reduce risk of cancer in patients with type-2 diabetes. (Evans et al. 2005).

The safety outcome of the present study also suggests that the two components (Atorvastatin and Metformin) of the FDC were well tolerated by the study subjects, as the adverse events observed in the study were mild to moderate in nature and resolved without sequelae.