5.0 DISCUSSION

Selective serotonin reuptake inhibitors (SSRIs) are antidepressants that block the reuptake of serotonin in the brain. Compared to the older tricyclic antidepressants (TCAs), SSRIs have less of an effect on histaminic and muscarinic receptors. The improved side effect profile leads to increased compliance with the SSRIs, their improved tolerability, as well as lower lethality in overdose, safety in cardiovascular disease, and lower incidence of weight gain, has resulted in the SSRIs becoming first-line agents for the treatment of depressive disorders (Antidepressant review, March 2013).

The sertraline is presumed to be linked to its inhibition of CNS neuronal uptake of serotonin (5HT) into human platelets. It has only very weak effects on norepinephrine and dopamine neuronal reuptake, and no significant affinity for adrenergic (alpha1, alpha2, beta), cholinergic, GABA, dopaminergic, histaminergic, serotonergic (5HT1A, 5HT1B, 5HT2), or benzodiazepine receptors; antagonism of such receptors has been hypothesized to be associated with various anticholinergic, sedative, and cardiovascular effects for other psychotropic drugs. Sertraline does not inhibit monoamine oxidase (Grimsley SR and Jann MW, 1992).

Sertraline show linear pharmacokinetics in that a change in dose leads to a proportional change in drug concentration. The effects of the other SSRIs, which have nonlinear pharmacokinetics, would be expected to increase disproportionately with higher doses (Antidepressant review, March 2013).

Bioavailability (BA) and bioequivalence (BE) studies play a major role in the drug development phase for both new drug products and their generic equivalents, and thus attract considerable attention globally. BE is a strategy to introduce generic equivalents of brand-name drugs (innovator drugs) to lower the cost of medication.

Life expectancy of patients has increased globally during the last three decades due to the new drug discovery (brand-name drugs) as well as generic drug production. It is well known that most health care interventions occur through medication. The rising cost of medication has been contributing to the total overall cost of health care and
thus receives considerable attention globally. A major strategy for lowering the cost of medication, and thereby reducing its contribution to total health care costs, has been the introduction of generic equivalents of brand-name drugs (innovator drugs).

BE studies are a critical component of Abbreviated New Drug Applications (ANDA). The purpose of these studies is to compare relative BA measures between a pharmaceutically equivalent multi-source test product and the corresponding reference pioneer product. The innovator product is termed as reference listed drug (RLD). Together with the determination of pharmaceutical equivalence, demonstrating BE allows a regulatory conclusion of therapeutic equivalence and interchangeability between the test and reference product [The Orange book, CDER, 2007].

The present study was undertaken to determine the rate and extent of sertraline between the test formulation, SERLIFT 100 (contains Sertraline hydrochloride equivalent to 100 mg sertraline) and the reference formulation, ZOLOFT (each tablet contains sertraline hydrochloride equivalent to 50 mg sertraline; total dose 100 mg) in healthy, adult, human subjects under fasting conditions and to assess the safety profile assess the safety of SERLIFT and ZOLOFT in healthy adult human subjects under fasting condition.

This section discusses the various aspects of the study including the methodology adopted and the results obtained. This section is divided into three parts: Clinical Phase, Bio-analytical Phase and Pharmacokinetic & Statistical Analysis.

**CLINICAL PHASE**

The principles of ICH: Good Clinical Practices (1996) were followed in the clinical study carried out in the present work. The study protocol and the informed consent form were reviewed and approved by the Jamia Hamdard Institutional Review Board which complied with the regulatory requirements. The study was designed based on the known pharmacokinetics of the study drug, sertraline and in compliance with the national and the international regulatory guidelines.
Successfully determining the bioavailability of generic drugs to their respective reference drugs depends mostly on design and managing the conduct of study such that the highest quality samples are obtained. Some regulatory authorities FDA, Europe, Canada Malaysia, Australia are providing specific information about Reference Listed Drug (RLD)/ reference product information on their websites, which makes it easy for investigators to proceed with BA/BE study design. So, specific attention should be paid to selecting as well as collecting the appropriate reference product details. Generally the study design and number of studies (single- dose and/or multiple-dose and/or fasting and/or fed) depend on the RLD or reference product, physico-chemical properties of the drug, its pharmacokinetic properties, and proportionality in composition with justification along with respective regulatory guidance and specifications.

The study was conducted using an open label, balanced, randomized, two-treatment, two-period, two-sequence, single-dose, crossover design in healthy, adult, human subjects under fasting conditions. Crossover designs are generally employed when intra-subject CV (approx. 15%) is usually substantially smaller than that inter-subject CV (approx. 30%). In Crossover design the treatments are compared on the same subject, the inter-subject variability does not contribute to the error variability. Subject randomization causes unbiased determination of treatment effects (FDA, 2003; CDSCO, 2005; Malaysia, 2000).

The study was carried out under fasting conditions. Fasting conditions are considered to be the most sensitive to detect a potential difference between formulations. For products where the product label recommends intake of the reference medicinal product on an empty stomach or irrespective of food intake, the study should be conducted under fasting conditions (EMA, 2010).

For fasting studies, EMA (2010) recommends that subjects should fast for at least 8 hours prior to drug administration and no food is allowed for at least 4 hours post dose. However CDSCO (2005) recommends that study should be conducted after an overnight fast (at least 10 hours) with a subsequent fast of 4 hours following dosing.
Accordingly, study was done after an overnight fast of at least 10 hours and subsequent fast of 4 hours following dosing.

The number of subjects required for a study should be statistically significant and should be sufficient to allow for possible withdrawals or removals (drop outs) from the study. The number of subjects required is determined by the error variance associated with the primary characteristic to be studied as estimated from a pilot experiment, from previous studies or from published data, the significance level desired, the expected deviation from the reference product compatible with BE (delta, i.e. percentage difference from 100%) and the required power (CDSCO, 2005; FDA, 2003; Malaysia, 2000). The sample size estimation in this present study based on available in-house study on Sertraline Tablets. Considering a Test / Reference ratio lying between 95-105% and an intra-subject CV of approximately 18%, 16 subjects may yield a power of 80% to show bioequivalence under bioequivalence assumptions. However, to be conservative and to allow for possible dropouts and/or withdrawals, 18 subjects were considered for this study.

Blood sampling is very vital in the design of the study. A sufficient number of samples to adequately describe the plasma concentration-time profile should be collected. For most drugs, blood samples should be drawn at appropriate times to describe the absorption, distribution, and elimination phases of the drug; 12–18 samples, including a predose sample, should be collected per subject per dose; should continue for at least 3 or more terminal half-lives of the drug (FDA, 2003; Malaysia, 2000).

The sampling schedule should include frequent sampling around predicted t_{max} to provide a reliable estimate of peak exposure. In particular, the sampling schedule should be planned to avoid C_{max} being the first point of a concentration time curve. The sampling schedule should also cover the plasma concentration time curve long enough to provide a reliable estimate of the extent of exposure which is achieved if AUC_{0-t} covers at least 80% of AUC_{0-∞} (EMA, 2010; CDSCO, 2005) recommends that the blood sampling should be extended to at least 3 elimination half lives; at least 3 sampling points during absorption phase, 3-4 at the projected T_{max}, and 4 points during elimination phase. Accordingly, sampling was done.
Exclusion criteria for the study were set after reviewing the safety profile of Sertraline. Sertraline is contraindicated in patients who are hypersensitive to any component of this medication, in patients with depression, other psychiatric disorders (including anxiety, agitation, panic attacks, insomnia, irritability, hostility aggressiveness, impulsivity, psychomotor restlessness, hypomania, mania etc.), and/or suicidal ideation, dizziness, tremor and/or seizure (Pfizer, 2010).

Subjects were judged to be medically healthy based on their medical history and demographic data (which included sex, age, height, weight and number of cigarettes smoked per day), physical examination, vital signs and laboratory tests for haematological parameters, biochemistry and renal functions, and disease markers for syphilis, HIV and hepatitis B and C and urine analysis.

The study was conducted in 18 Asian males and the mean age, weight, height and BMI of the study subjects were 29.78 years (range 21 to 42 years), 59.18 kg (range 45.5 to 70.7 kg), 165.95 cm (range 158.3 to 178.6 cm) and 21.48 kg/m$^2$ (range 18.16 to 25.14 kg/m$^2$) respectively. In general, subjects should be between 18 – 55 years old, capable of giving informed consent and of weight within the normal range according to accepted life tables or Body Mass Index (BMI) of 18 – 30 kg/m$^2$. They should be screened for suitability by means of clinical laboratory tests, review of medical history, and a comprehensive medical examination. (Malaysia, 2000; FDA, 2003; EMA, 2010).

The test and reference products should be administered with a standardized volume of fluid as fluid intake may influence gastric passage for oral administration forms (EMA, 2010). US FDA (2003) recommends that the drug should be administered with about 8 ounces (240 mL) of water while EMA (2010) states that it should be taken with at least 150 mL water, Health Canada (2012) recommends 150-250 mL. It is recommended that water is allowed as desired except for one hour before and one hour after drug administration (US FDA, 2003; EMA, 2010). Accordingly, the drug was administered with 240 mL of water and water was not allowed from 1 hour before dosing until 2 hours post-dose.
The treatment periods should be separated by a washout period sufficient to ensure that there is no pre-dose concentration at the beginning of second period. Washout period should be equal to at least 5 elimination half lives (US FDA, 2003; CDSCO, 2005; EMA, 2010). In this study a washout period of twenty one days was given between dosing of each period, much beyond 5 elimination half lives of the drug. No pre-dose concentrations were observed in any of the subjects at the beginning of second period, confirming that the washout period selected was adequate.

Safety of the subjects was assessed throughout the study by recording vital signs, clinical examination and adverse event monitoring. Vital signs and Laboratory investigations for hematology and biochemistry were also performed at the last ambulatory visit. Vital signs (oral temperature, sitting blood pressure and radial pulse), clinical examination were found to be normal for all the completed subjects during the course of the study.

The study treatments were well tolerated by the study subjects. Adverse event monitoring was done throughout the study. Two adverse events including one laboratory adverse event was reported during the study. One adverse event of nausea was reported post-dose period II in reference group. The event had possible relationship to the study drug and recovered without sequelae. The event was mild and not serious in nature. One laboratory adverse event of raised eosinophils was reported at the end of study safety assessment in test group. The event had unlikely relationship to the study drug and recovered without sequelae. The event was moderate and not serious in nature.

There were no serious adverse events during the conduct of the study. These results were in agreement with the safety profile reported in literature for single dose administration of sertraline (Pfizer, 2012). In a bioequivalence study conducted by (Suvimol Niyomnaitham et al 2009) similar tolerability and safety profile was reported for sertraline.
Bio-analytical Phase

Liquid chromatography/tandem mass spectrometry (LC-MS/MS) is a highly specific and sensitive analytical technique that has become the industry standard for quantifying drugs, metabolites, and endogenous compounds in biological matrices (e.g. plasma). The technique is widely used because of its ability to accurately quantitate analytes of interest with minimal sample clean-up and rapid LC separation.

Various analytical methods have been reported for the analysis of sertraline in biological samples such as capillary electrophoresis (D. Schaller et al., 2006), gas chromatography-mass spectrometry (GC–MS) (S.M.R. Wille et al., 2005), high-performance liquid chromatography (HPLC) with fluorescence (E. Lacassie et al., 2000), diode array and UV detection (C. Frahnert et al., 2003), and LC–MS–MS (H. Kirchherr et al., 2006)

In this study for the determination of sertraline in human K3EDTA plasma using sertraline-d3 as internal standard a high performance liquid chromatographic tandem mass spectrometric method was developed.

The separation was performed on Discovery C8 (50 x 4.6 mm, 5µm) column using Acetonitrile: Buffer-1 (40:60, v/v) as mobile phase. Sample preparation process was performed by solid phase extraction technique. Solid phase extraction gives greater reproducibility and cleaner extracts as compared with other techniques like protein precipitation and liquid - liquid extraction (Sharma et al., 2010).

Bioanalytical method validation includes all of the procedures that demonstrate that a particular method used for quantitative measurement of analytes in a given biological matrix, such as blood, plasma, serum, or urine, is reliable and reproducible for the intended use. The fundamental parameters for this validation include (1) accuracy, (2) precision, (3) selectivity, (4) sensitivity, (5) reproducibility, and (6) stability (US FDA, 2001a). In the present study the method was validated in terms of selectivity, sensitivity, precision, accuracy, linearity, recovery, dilution integrity, ruggedness and stability studies. Stability studies included freeze-thaw, bench top, in-injector, short term, long term and stock solution. Before the initiation of unknown plasma samples
of the biostudy, validation was completed and all validation parameters met the acceptance criteria. The acceptance criteria of international regulatory standards were followed.

Selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample (US FDA, 2001a). Selectivity using ten lots of normal human plasma, one lot of haemolysed human plasma and one lot of lipemic plasma was evaluated and none showed significant interfering peaks at the retention time of the drug and the internal standard. The precision of an analytical method describes the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of biological matrix. The accuracy of an analytical method describes the closeness of mean test results obtained by the method to the true value (concentration) of the analyte (US FDA, 2001a).

The method was precise and accurate as was confirmed by three precision and accuracy batches. Within batch precision and accuracy ranged from 0.6% to 4.6% and 94.2% to 104.2% respectively. Between batch precision and accuracy ranged from 1.4% to 4.6% and 97.6% to 102.0% respectively. These results were within the acceptance limits as per the regulatory guidelines. Recovery pertains to the extraction efficiency of an analytical method within the limits of variability (US FDA, 2001a). Recovery of sertraline was found to be 72.03%.

Drug stability in biological fluid is a function of the storage conditions, the chemical properties of the drug, the matrix, and the container system. Stability procedures should evaluate the stability of the analytes during sample collection and handling, after long-term (frozen at the intended storage temperature) and short-term storage, and after going through freeze and thaw cycles and the analytical process (US FDA, 2001a). The results of all the stability studies conducted for sertraline met the acceptance criteria. All validation parameters met the acceptance criteria. So, the method was reliable, reproducible and accurate.

The validated method was used for the analysis of subject samples. EMA (2010) recommends that a calibration curve should be generated for each analyte in each
analytical run and it should be used to calculate the concentration of the analyte in the unknown samples in the run. A sufficient number of separately prepared Quality Control samples should be analysed with processed test samples at intervals based on the total number of samples. In the present study, 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 the subject samples. The final concentration data was used for pharmacokinetic and statistical analysis.

**Pharmacokinetic and Statistical Analysis**

In studies to determine bioequivalence after a single dose, pharmacokinetic parameters, AUC$_{t}$, AUC$_{\infty}$, C$_{\text{max}}$ and t$_{\text{max}}$ should be determined. Additional parameters that may be reported include the terminal rate constant, $\lambda_z$, and t$_{1/2}$ (EMA, 2010).

In the present study pharmacokinetic analysis was performed using standard non-compartmental methods in WinNonlin®, Version 5.0.1, software. The mean pharmacokinetic parameters of T$_{\text{max}}$ (hrs), C$_{\text{max}}$ (ng/mL), AUC$_{0-t}$ (ng.hr/mL), AUC$_{0-\infty}$ (ng.hr/mL) after administration of test and reference formulations of sertraline.

Pharmacokinetic data obtained from this study revealed that mean maximum concentration (C$_{\text{max}}$) in plasma after administration of reference product R and test product T were 35.72 (±6.30) ng/mL and 34.78 (±8.77) ng/mL.

These results indicate that the mean maximum concentrations (C$_{\text{max}}$) of the test and reference products are comparable. The mean area under the curve, AUC$_{0-t}$ and AUC$_{0-\infty}$ were 1186.70 (±356.93) hr*ng/mL and 1254.93 (±402.63) hr*ng/mL, for the reference product, R. For test product T the area under the curve (AUC$_{0-t}$) were 1161.27 (±404.03) hr*ng/mL and AUC$_{0-\infty}$ was 1228.29 (±453.83) hr*ng/mL.

These findings are consistent with the results reported by MHRA, (Public Assessment Report 2013). These values are in agreement with those reported in the literature (Pfizer 2010). The mean area under the curve, AUC$_{0-t}$ and AUC$_{0-\infty}$ were 914.52 hr*ng/mL and 1045.7 hr*ng/mL for reference product and for test product T the area under the curve (AUC$_{0-t}$) were 946.86 hr*ng/mL and 1112.07 hr*ng/mL.
Statistical analysis was performed using SAS® system for Windows, release 9.1.3 (SAS Institute Inc., USA). For test (T) and reference (R) formulations the percentage point estimate ratio of the least square means and its 90% confidence interval for the log transformed pharmacokinetic parameters $C_{max}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ were calculated.

To meet the assumption of normality (all biological data follow log normal distribution) of data underlying the statistical analysis, the logarithmic transformation was carried out for the pharmacokinetic parameters $C_{max}$ and AUC before performing statistical analysis (EMA, 2010; CDSCO, 2005, US FDA, 2003). The log-transformed pharmacokinetic parameters $C_{max}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ were analyzed using a mixed effects ANOVA model using Type III sum of squares with the main effects of sequence, period and treatment as fixed effects and subjects nested within sequence as random effect. The sequence effect was tested at the 10% level of significance using the subjects nested within sequence mean square as the error term and treatment and period effects were tested at the 5% level of significance against the residual error (mean square error) from the ANOVA model as the error term. Based on the ANOVA results, no significant treatment, period and sequence effect was observed for log transformed pharmacokinetic parameters $C_{max}$, $AUC_{0-t}$ and $AUC_{0-\infty}$.

The assessment is based upon 90% confidence intervals for the ratio of the averages (population geometric means) of the measures ($C_{max}$ and AUC) for the test and the reference products. This method is equivalent to two one-sided tests with the null hypothesis of bioinequivalence at the 5% significance level. To establish bioequivalence, the calculated confidence interval should fall within a BE limit, usually 80-125% for the ratio of the product averages (Malaysia, 2000; US FDA, 2001b; EMA, 2010; CDSCO, 2005).

In the present study the T/R (%) ratios for log transformed data for the pharmacokinetic parameters $C_{max}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ were 96.10, 96.36 and 96.27 respectively. The 90% confidence intervals for log transformed data for $C_{max}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ for the test product T vs. reference R (T/R) were 87.14-105.97, 88.27-
105.19 and 88.18-105.10 respectively, which were within the stated regulatory limit of 80-125%.

These results show that the rate of drug absorption (\(C_{\text{max}}\)) and the extent of drug absorption (AUC) of both the test products T and reference product R are comparable.

The intra subject variability for the \(C_{\text{max}}\), AUC\(_{0-t}\), and AUC\(_{0-\infty}\) was reported for log-transformed data. Overall, the intrasubject variability (expressed as % CV) for all the products was less than 30%, indicating low variability. Intra subject variability was 16.3, 14.6 and 14.7 for \(C_{\text{max}}\), AUC\(_{0-t}\) and AUC\(_{0-\infty}\) respectively. Power of the test for \(C_{\text{max}}\), AUC\(_{0-t}\), and AUC\(_{0-\infty}\) was found to be 95.93%, 98.29% and 98.28% respectively.

Based on the above discussion, it can be concluded that the SERLIFT 100 mg (contains Sertraline hydrochloride equivalent to 100 mg sertraline) of Ranbaxy is bioequivalent to the reference formulation, ZOLOFT (contains sertraline hydrochloride equivalent to 50 mg sertraline; total dose 100 mg) of Pfizer and the test and reference formulations are well tolerated by the study subjects.