Statistical Analysis Plan: February 2018

An open label, balanced, randomized, single-dose, two-treatment, two-sequence, twoperiod, crossover oral drug-drug interaction study of spironolactone (perpetrator) and Digoxin (substrate drug) in healthy adult human subjects under fasting condition.

PHARMACOKINETIC ASSESSMENTS:

Concentration data of subjects versus time, which are received after analysis of samples, will be included in the final data analysis. Data from subjects with missing concentration values (missed blood samples, lost samples, samples unable to be quantified) may be used if pharmacokinetic parameters can be estimated using remaining data points, otherwise data from these subjects will be excluded from the final analysis.

All concentration values below the limit of quantification (BLQ) will be set to zero for all pharmacokinetic and statistical calculations. Any missing samples will be reported as 'Missing' and will be denoted as "M" while running pharmacokinetic and statistical analysis.

The following pharmacokinetic parameters will be computed using noncompartmental model of Phoenix WinNonlin Professional Software Version 6.4 (Pharsight Corporation, USA) or above.

Pharmacokinetic Parameters for digoxin in plasma:

Primary Pharmacokinetic parameters

Cmax : Maximum measured plasma concentration.

AUC0-96 : Area under the plasma concentration versus time curve from time 0 to the 96 hour time point concentration, calculated by linear trapezoidal method.

Secondary Pharmacokinetic parameters

Tmax : Time to achieve maximum plasma concentration. If the maximum value occurs at more than one time point, Tmax is defined as the first time point with this value.

Pharmacokinetic Parameters for digoxin in urine:

CLR: Renal clearance Ae0−240h/AUC0−240 h

fe: Unchanged drug excreted in urine

For all the above computations, actual time points of the sample collection will be used in case of sample collection deviations.

In clinical phase or sample analysis process, if 3 or more consecutive post dose samples for any subject are found to be missing, samples of such subjects will be analyzed but pharmacokinetic data of those subjects will be excluded from the final statistical analysis.

Pharmacokinetic data of those subjects will be tabulated and reported in the study report as a separate table. However, the data of such subjects will also be considered for statistical analysis if the 3 consecutive post dose samples are the last three samples of elimination phase.

If the pre-dose concentration appears to be > 5% of the Cmax in any subject at any period, the data of such a subject will be excluded from the statistical analysis.

STATISTICAL ANALYSIS

Statistical analysis will be performed by using SAS statistical software version 9.3 or above.

Descriptive statistics of all the pharmacokinetic parameters will be computed and reported for digoxin.

The log-transformed pharmacokinetic parameters Cmax and AUC0-96 were analysed using Type III sum of squares, with the main effects of formulation, period, sequence and subjects nested within sequence as random effect.

Statistical analysis was performed using SAS® package (SAS Institute Inc., USA, Version 9.3).

The presence or absence of DDI between the two treatments (A and B) was concluded based on the statistical results of 90% confidence interval for the ratio of the geometric least squares mean for log-transformed pharmacokinetic parameters Cmax and AUC0-96 for Digoxin. Absence of drug-drug interaction would be established if the 90% CI for the ratio of population geometric means of log-transformed data of Cmax and AUC0-96 between two treatments (A and B), were in the equivalence limits of 80.00 - 125.00% for Digoxin.

Renal clearance (CLR)/ Percent Recovered and unchanged drug excreted in urine (fe)/Amount Recovered for Digoxin in the presence and absence of spironolactone was provided as supportive data.