CLINICAL STUDY REPORT

1. TITLE PAGE

Study title	An open label, balanced, randomized, single-dose, two-treatment, two-sequence, two-period, crossover oral drug-drug interaction study of spironolactone (perpetrator) and Digoxin (substrate drug) in healthy adult human subjects under fasting condition.
Treatment (A)	LANOXIN® (digoxin) USP 250 mcg (substrate drug), manufactured for Concordia Pharmaceuticals Inc., St. Michael, Barbados BB11005. Initial U.S
Treatment (B)	LANOXIN® (digoxin) USP 250 mcg (substrate drug) + Spironolactone Oral Suspension 100 mg (Perpetrator; Carospir® Oral Suspension 20 mL of 25 mg / 5 mL of CMP Development LLC, USA.)
Indication studied	Not Applicable
Study design	An open label, balanced, randomized, single-dose, two-treatment, two-sequence, two-period, crossover, drug-drug interaction study in healthy adult human subjects under fasting condition.
Name of the sponsor	CMP Development, LLC.
Protocol No.	C-CMP-SPI-001
Development phase of study	Drug-Drug interaction study
Study start date	10 Mar 2019
Study end date	17 Apr 2019 (last blood sample collection of the study)
Principal Investigator	Dr. P. Venkatesh, MBBS, Ph.D Clinical Pharmacology Department, ClinSync Clinical Research Pvt. Ltd., JSR Mall, Plot No.7 to 18, Madinaguda Village, Serilingampally Mandal, Hyderabad-500050, Telangana, India Phone No.: 040-29887005/06 Email ID: venkatesh.p@clinsynccro.com

Sponsor's Representative	Gerald Sakowski Chief Executive Officer CMP Development LLC PO Box 147 8026 US Highway 264A Farmville, NC 27828 Telephone: (800)227-6637
Version	01
Date of study report	01 Jul 2019
This study was performed according to the protocol and in compliance with Good Clinical Practices (GCP) and Good Laboratory Practices (GLP)	

2. SYNOPSIS

Name of Sponsor / Company: CMP Development LLC	**Individual Study Table Referring to Part of the Dossier**	***(For National Authority Use only)***
Name of Finished Products: Digoxin USP 250 mcg + Spironolactone suspension 100 mg (20 mL of 25 mg / 5 mL)	**Volume:** **Page:**	
Name of Active Ingredients: Digoxin		
Title of Study	An open label, balanced, randomized, single-dose, two-treatment, two-sequence, two-period, crossover oral drug-drug interaction study of spironolactone (perpetrator) and Digoxin (substrate drug) in healthy adult human subjects under fasting condition.	
Investigators	**Principal Investigator:** Dr. P. Venkatesh, MBBS **Sub Investigator:** Dr. Sreedhar Reddy Peddi, MD General Medicine, DNB (cardiology) **Clinical Investigator:** Dr. S. Ravikishore, MBBS **Bioanalytical Investigator:** Mr. Praveen Rao M, M. Sc., **Pharmacokinetic Investigator:** Dr. Jaganmohan S, Ph.D, PDF **Statistical Investigator:** Mr. Tushar Shinde, M. Sc **Note:** Dr. P.Venkatesh handed over his Principal Investigator resposibilities to Dr.S.Ravikishore on Period I check-in day due to his personal reasons and the same information was notified to EC and sponsor. For Period II,	

	subsequent follow up and approval of has been done by Dr.Venkatesh as study Principal Investigator. This information is also applicable to the section 6.0	
Study Center	ClinSync Clinical Research Pvt. Ltd., Clinical Pharmacology Department, JSR Mall, Plot No.7 to 18, Madinaguda Village, Serilingampally Mandal, Hyderabad-500050, Telangana, India Phone No.: 040-29887005/06	
Publication (reference)	Not Applicable	
Bioanalytical, Pharmacokinetic and Statistical Facility	ClinSync Clinical Research Pvt. Ltd., Clinical Pharmacology Department, JSR Mall, Plot No.7 to 18, Madinaguda Village, Serilingampally Mandal, Hyderabad-500050, Telangana, India Phone No.: 040-29887005/06	
Clinical start date	10 Mar 2019	
Clinical end date	17 Apr 2019 (last blood sample collection of the study)	
Bioanalytical analysis start date	**Plasma**	23 Apr 2019
	Urine	24 May 2019
Bioanalytical analysis end date	**Plasma**	04 May 2019
	Urine	04 Jun 2019
Statistical analysis start date	07 Jun 2019	
Statistical analysis end date	10 Jun 2019	
Phase of development	Drug-Drug interaction study	
Objectives	**Primary Objective:** To characterize the pharmacokinetic profile of Digoxin (substrate drug) in the presence and absence of spironolactone (perpetrator) in healthy adult human subjects under fasting condition. **Secondary Objectives:** • To assess whether there is a drug-drug interaction with	

	concomitant use of spironolactone. • To measure renal clearance (CLR)/Percent Recovered and unchanged drug excreted in urine (fe)/ Amount Recovered for digoxin in the presence and absence of spironolactone (perpetrator) in healthy adult human subjects under fasting condition. • To monitor safety of the subjects in drug-drug interaction study.
Methodology	This was an open label, balanced, randomized, single-dose, two-treatment, two-sequence, two-period, crossover, drug-drug interaction study in healthy adult human subjects with at least 28 days washout period between each treatment period under fasting condition. In both periods (Period-I and II), from Day 1 to Day 5 and Day 7 to Day 9, the subjects who were randomized to treatment B, were administered Spironolactone Oral Suspension 100 mg (Perpetrator; Carospir® Oral Suspension 20 mL of 25 mg / 5 mL), and the subject who were randomized to treatment A were not dosed. On all these days the standard breakfast was served 0.50 hr (30 min) post dose. On Day 6, after overnight fasting for at least 10.00 hours the subjects were dosed either with the Treatment (A) [LANOXIN® (digoxin) USP 250 mcg (substrate drug)] or Treatment (B) [LANOXIN® (digoxin) USP 250 mcg (substrate drug) + Spironolactone Oral Suspension 100 mg (Perpetrator; Carospir® Oral Suspension 20 mL of 25 mg / 5 mL)] as per the randomization scheme. Urine & Blood samples collection were collected after the completion of dosing on Day 6. **Urine samples** A total of 11 urine samples were collected from each subject in each study period at intervals of (0.00-1.00), (1.00-2.00), (2.00-4.00), (4.00-6.00), (6.00-8.00), (8.00-12.00), (12.00-16.00), (16.00-24.00), (24.00-48.00), (48.00-72.00) and (72.00-96.00) hours post dose. Immediately out of the total volume of urine collected at each collection time interval, approximately 10 mL (2 x 5 mL aliquots) of urine samples were transferred into two pre-labeled containers for analysis and stored at deep

	freezer maintained at -20°C or lower until dispatched from Clinical Unit to Bioanalytical Department for analysis. After collecting the urine samples from all the subjects at each collection time interval, the total pooled volume was recorded and transferred to analytical site for analysis after the completion of clinical phase. **Blood samples** A total of 25 (1 × 5 mL) blood samples were collected from each subject in each study period at pre-dose (0.00) and at 0.25, 0.50, 0.75, 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00, 36.00, 48.00, 60.00, 72.00 and 96.00 hours post dose. **Note:** Blood samples up to 3 hours post dose were collected at bed side. The pre and post-dose blood samples up to 24.00 hrs were collected via an indwelling cannula and 36.00, 48.00, 60.00, 72.00, and 96.00 post dose samples were collected by a fresh vein-puncture. The pre-dose blood sample were collected within 2 hours before dosing and the post-dose in-house samples were collected with a window period of + 2 minutes from the scheduled sampling time. The collected blood samples were kept on ice-packs until centrifugation. All the samples except 0.25 hr sample in period I, were centrifuged within 45 minutes from the scheduled blood sample collection. The samples were centrifuged at the set conditions of 4000 rpm for 10 minutes at 4°C to separate plasma. The separated plasma was transferred to pre-labeled polypropylene tubes in two equal aliquots. The total amount of blood collected from each subject during the study was about 292 mL Subjects were abstained from consuming any alcoholic products from 48.00 hours prior to check-in to till check-out / last sample of the study. They were not allowed to have any xanthine-containing food and/or beverages (like chocolate, tea, coffee, cola drinks), cigarettes and tobacco containing products and grapefruit and/or it's juice from 48.00 hours prior to check-in to till check-out / last sample of the study.

	On Day 6, subjects were fasted for at least 10.00 hours prior to dosing. Water was provided ad libitum during the study, except one hour before and one hour after dosing on Day 6. On Day 6, subjects who are dosed remained in a supine position or semi recumbent position for 03 hours post-dose and only necessary movement was allowed during this period. Thereafter subject was allowed to ambulate freely during the remaining part of the study. Subject did not sit down (except as directed by the physician secondary to adverse events) during restriction period.
Number of subjects	• No. of subjects planned: 28 + 04 (standby subjects) • No. of subjects randomized: 28 • No. of subjects dosed in period I: 28 • No. of subjects dosed in period II: 28 • No. of subjects withdrawn: 00 • No. of subjects dropped out: 00 • No. of subjects completed the study: 28 • No. of subjects analyzed in bioanalytical facility: 28 • No. of subjects analyzed for pharmacokinetic and statistical analysis: 28
Diagnosis and main criteria for inclusion	Healthy consenting human adult subjects between 20 and 35 years of age (both ages inclusive), of Body Mass Index (BMI) within 18.5 to 24.9 Kg/ m^2 with normal laboratory measurements were included in the study. Subjects weighing less than 60 kg were not enrolled.
Treatment (A)	LANOXIN® (digoxin) USP 250 mcg (substrate drug), manufactured for Concordia Pharmaceuticals Inc., St. Michael, Barbados BB11005. Initial U.S
LANOXIN® USP 250 mcg Lot No.	AH3690B
Dose and mode of administration	Single oral dose administered with 120 mL of water under fasting condition.
Treatment (B)	LANOXIN® (digoxin) USP 250 mcg (substrate drug) manufactured for Concordia Pharmaceuticals Inc., St. Michael, Barbados BB11005. Initial U.S + Spironolactone Oral Suspension 100 mg (Perpetrator; Carospir® Oral

	Suspension 20 mL of 25 mg / 5 mL of CMP Development LLC, USA)
(Carospir® Oral Suspension) Lot No.	1118211
Dose and mode of administration	In case of subjects who were assigned Treatment A, Single oral dose of LANOXIN® (digoxin) USP 250 mcg was administered with 240 ± 2 mL of water under fasting condition. Subjects swallowed the tablet as a whole. Dosing compliance was assessed by oral cavity check. In case of subjects who were assigned Treatment B, single oral dose of LANOXIN® (digoxin) USP 250 mcg was administered with 120 mL of water under fasting condition and Carospir® Oral Suspension 100 mg (20 mL of 25 mg / 5 mL) was administered with 120 mL of water with a graduated syringe. Dosing activity was done under the supervision of cardiologist. CaroSpir oral suspension administration procedure 20 mL of 25 mg / 5 mL of Carospir® Oral Suspension was loaded in the syringes well in advance prior to dosing on the day of dosing. The suspension in the syringe was directly administered to the subjects. Then the syringe was rinsed with approximately 10 mL from 120 mL of dosing water and administered to the subjects. This rinsing procedure was done until the syringe was free of the contents of the medication. Then the remaining quantity of water from the assigned 120 ml water was finally administered to the subjects.
Duration of the treatment	A single oral dose of Carospir® 100 mg Oral (Spironolactone 20 mL of 25 mg / 5 mL) suspension from Day 1 to Day 5 and Day 7 to Day 9 and on Day 6 either LANOXIN® (digoxin) USP 250 mcg (substrate drug) or LANOXIN® (digoxin) USP 250 mcg (substrate drug) + Carospir® 100 mg Oral (Spironolactone 20 mL of 25 mg / 5 mL) suspension (Perpetrator) were administered on 11 Mar 2019 and 08 Apr 2019 in period I and period II, respectively separated by a washout period of 28 days. Total duration of the study from the check in of period I to the last blood draw of period II did not exceed 39 days.
Criteria for evaluation: Safety Evaluation	Subjects were monitored for safety and tolerability during the entire study. The subject's Clinical examination along

	with vital signs (seated blood pressure, radial pulse rate and oral/aural temperature) were measured during check-in and check-out of each period. Clinical examination was done on prior to dosing on Day 06 in each study period. Oral/aural temperature was measured prior to dosing in each study period. Vital parameters – seated blood pressure and radial pulse rate were measured at prior to dosing, 1.00, 3.00, 5.00 and 11.00 hours post dose in each period from Day 1 to Day 5, and from Day 6 to Day 9, vitals were measured prior to dosing, at 1.00, 2.00, 3.00, 5.00, 8.00 and 11.00 hours post dose in each period. **Note** • On Day 6 the Vital signs (i.e. up to 3.00 hours post dose) was performed at bed side. • For subjects who were randomized to treatment A, the vitals were checked at 5.00 hours post dose during day 1 to Day 5. ECG was performed at 1.00 and 3.00 hours post dose in each period on Day 6, before period II check-in for subject study eligibility and at check-out of each period of the study. 2D echo was performed at the time of screening. In each study period subject's creatinine clearance and serum potassium levels were measured on Day 5 for the dosing eligibility on Day 6. For the safety of the subjects, hematology (except blood grouping and Rh typing) and biochemistry (except random blood sugar) investigations were repeated at the end of the study.
Pharmacokinetic Evaluation	The following pharmacokinetic parameters for Digoxin were obtained using non-compartmental method (WinNonlin, version 8.1, Pharsight Corporation, USA): ➢ C_{max}, AUC_{0-96}, and t_{max} using plasma data, ➢ Renal clearance (CLR) /Percent Recovered, Unchanged drug excreted in urine (fe)/Amount Recovered using urine data.
Statistical Evaluation	The log-transformed pharmacokinetic parameters C_{max} and AUC_{0-96} 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 C_{max} and AUC_{0-96} 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 C_{max} and AUC_{0-96} between two treatments (A and B), were in the equivalence limits of 80.00 - 125.00% for Digoxin.

Summary and Conclusions

Pharmacokinetic & Statistical Results:

Table 1: Summary of Plasma Pharmacokinetic parameters for digoxin – Treatment (A)

Treatment	Product/Statistics	C_{max} (ng/mL)	AUC_{0-96} (ng.hr/mL)	T_{max} (hr)
Treatment (A)	N	28	28	28
	Mean	1530.188	13715.974	0.744
	SD	502.810	2669.003	0.195
	Min	644.613	8632.172	0.500
	Median	1429.822	13440.345	0.750
	Max	2734.608	21455.761	1.330
	Geometric Mean	1456.085	13473.043	0.721
	CV% Geometric Mean	32.993	19.431	25.996

Table 2: Summary of Plasma Pharmacokinetic parameters for digoxin – Treatment (B)

Treatment	Product/Statistics	C_{max} (ng/mL)	AUC_{0-96} (ng.hr/mL)	T_{max} (hr)
Treatment (B)	N	28	28	28
	Mean	2432.908	16551.500	0.901
	SD	870.562	4108.500	0.569
	Min	539.707	6373.657	0.500
	Median	2422.385	17151.091	0.750
	Max	5044.130	23997.624	3.500
	Geometric Mean	2254.118	15996.644	0.810
	CV% Geometric Mean	45.789	28.453	44.310

Table 3: Ratio and 90% Confidence Intervals of Treatment (A) versus Treatment (B) for digoxin

PK Parameters (Unit)	Geometric Least Square Means and It's Ratio			ISCV (%)	90% CI	Power (%)
	Treatment A	Treatment B	(Treatment A / Treatment B) (%)			
LOG C_{max} (ng/mL)	1456.085	2254.118	64.597	40.07	54.18% to 77.02%	67.47
LOG AUC_{0-96} (ng.hr/mL)	13473.043	15996.644	84.224	18.56	77.45% to 91.59%	99.56

Table 4: Summary of Urine Pharmacokinetic parameters for digoxin – Treatment (A)

Treatment	Product/Statistics	Renal clearance (CLR)/Percent Recovered	unchanged drug excreted in urine (fe)/ Amount Recovered
Treatment A	N	28	28
	Mean	54.687	136717.72
	SD	29.158	72895.492
	Min	22.180	55449.800
	Median	46.111	115277.98
	Max	147.642	369104.30
	G. Mean	49.042	122606.02
	G.mean %CV	48.257	48.257

Table 5: Summary of Urine Pharmacokinetic parameters for digoxin – Treatment (B)

Treatment	Product/Statistics	Renal clearance (CLR)/Percent Recovered	unchanged drug excreted in urine (fe)/ Amount Recovered
Treatment B	N	28	28
	Mean	53.378	133446.07
	SD	21.256	53141.044
	Min	21.641	54103.200
	Median	51.185	127962.08
	Max	95.602	239003.90
	G. Mean	49.410	123523.87
	G.mean %CV	42.201	42.201

Safety Results	There were no adverse events recorded during the study. Therefore, the safety of the digoxin in the presence and absence of spironolactone would be considered as comparable.

Conclusion	The exposure (C_{max} and AUC_{0-96}) of digoxin was not comparable for the Treatment A and Treatment B. The geometric mean C_{max} was 1456.085 and 2254.118 ng/mL for Treatment A and Treatment B respectively. The geometric mean AUC_{0-96} was 13473.043 and 15996.644 ng.hr/mL for Treatment A and Treatment B respectively By comparing C_{max} and AUC_{0-96} plasma values of Treatment A and Treatment B, we conclude that these values were increased by 35% and 16% respectively for Treatment B. The %Treatment A/Treatment B ratio of digoxin was 64.597% and 84.224% for C_{max} and AUC_{0-96} respectively. The lower and the upper limits at 90% confidence interval were 54.18% - 77.02% and 77.45% - 91.59% for C_{max} and AUC_{0-96} respectively, which were not within the standard equivalence limit of 80.00-125.00%. Based on the above results presence of drug-drug interaction is established between digoxin and spironolactone. The Renal clearance (CLR)/Percent Recovered and unchanged drug excreted in urine (fe)/ Amount Recovered of digoxin was comparable for the Treatment A and Treatment B. The geometric mean Renal clearance (CLR)/Percent Recovered was 49.042 and 49.410 for Treatment A and Treatment B respectively. The geometric mean unchanged drug excreted in urine (fe)/ Amount Recovered was 122606.02 and 123523.87 for Treatment A and Treatment B respectively. By comparing these values, we conclude that there is not much difference between Treatment A and Treatment B, with respect to Renal clearance (CLR)/Percent Recovered and unchanged drug excreted in urine (fe)/ Amount Recovered.
DATE OF THE REPORT	01 Jul 2019

3. TABLE OF CONTENTS

List of Tables

List of Graphs

4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

%	Percentage
°C	Degree centigrade
ADR	Adverse Drug Reaction
AE	Adverse Event
ALT	Alanine Amino Transferase
ANOVA	Analysis of Variance
AST	Aspartate Amino Transferase
AUC_{0-96}	Area under the plasma concentration versus time curve from time 0 to the 96 hour time point concentration.
BLQ	Below Limit of Quantification
BMI	Body Mass Index
CDSCO	Central Drugs Standard Control Organization
CLR	Renal Clearance
C_{max}	Maximum measured plasma concentration
COA	Certificate of Analysis
CP	Clinical Pharmacology
CRF	Case Report Form
CRO	Contract Research Organization
CSR	Clinical Study Report
DDI	Drug-Drug Interaction
ESR	Erythrocyte sedimentation rate
fe	Unchanged drug excreted in urine
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
hr/hrs	Hours
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICMR	Indian Council of Medical Research
ICU	Intensive Care Unit
ID	Identity Document
IEC	Institutional Ethics Committee
IP	Investigational Product

IU	International Unit
K_2EDTA	Di potassium Ethylene Diamine Tetraacetic Acid
kg	kilogram
LC-MS/MS	Liquid Chromatography tandem mass spectrometry
Ln	Natural Logarithm
LOQ	Limit of Quantification
Ltd.	Limited
M	Missing samples
Mcg	Microgram
mg	Milligram
mL	Millilitre
MSE	Mean Square Error
No.	Number
NR	Not reportable
NS	Non significant
OTC	Over The Counter
P/A view	Posterior-Anterior view
PI	Principal Investigator
PK	Pharmacokinetic
RBC	Red Blood Corpuscles
Rh	Rhesus
rpm	Rotations per minute
SAE	Serious Adverse Event
SAS	Statistical Analysis System
SD	Standard Deviation
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SOP	Standard Operating Procedure
T_{max}	Time to achieve maximum plasma concentration
VDRL	Venereal Disease Research Laboratory
WBC	White Blood Corpuscles
βhCG	Beta Human Chorionic Gonadotropin

5. ETHICS

5.1 INSTITUTIONAL ETHICS COMMITTEE (IEC)

The final protocol No. C-CMP-SPI-001, Version No. 02 dated 07 Aug 2018 and other protocol related documents were reviewed and approved prior to study start by an Institutional Ethics Committee (IEC) as per below table.

Document Name	Document No. and Date	Submission date	Approval Date
Protocol	Version No. 02; dated 07 Aug 2018	22 Aug 2018	01 Oct 2018
Informed Consent Form (English)	Version No.: 02; dated 07 Aug 2018		
Informed Consent Form (Telugu)	Version No.: 02; dated 07 Aug 2018		
Case Report Form	Version No.: 02; dated 13 Aug 2018		
Other supporting documents			
Amendment to the protocol	Amendment No.: 01; dated 19 Dec 2018	21 Dec 2018	22 Dec 2018
Informed Consent Form (English)	Version No.: 03; dated 19 Dec 2018		
Informed Consent Form (Telugu)	Version No.: 03; dated 19 Dec 2018		

A list of the IEC members along with the IEC approval letter has been provided in Appendix 16.1.3.

5.2 ETHICAL CONDUCT OF THE STUDY

This study was conducted in compliance with the final protocol No. C-CMP-SPI-001 Version No. 02 dated 07 Aug 2018 and Amendment No 01 dated 19 Dec 2018, the applicable International Conference on Harmonization Guidelines for Good Clinical Practice (GCP), the relevant sections of Good Laboratory Practice (GLP), local laws and regulations (ICMR Guidelines on Biomedical Research, Schedule Y (amended version, 2014) of CDSCO (Central Drugs Standard Control Organization), relevant sections of Drugs and Cosmetics (First Amendment) Rules 2013, CDSCO Bioavailability & Bioequivalence guidelines, applicable USFDA guidelines, the provisions of Declaration of Helsinki (Brazil, October 2013) and applicable in- house SOP's.

5.3 SUBJECT INFORMATION AND CONSENT

Prior to screening of the volunteers, the screening consent form was provided as per the in-house SOP. Volunteers who were willing to participate in the screening process personally gave their consent by affixing their signature and date in the appropriate screening consent form. The volunteers were screened only after they voluntarily consented for the screening process.

The study informed consent form was submitted with the protocol for review and approval by the ethics committee. The eligible volunteers were contacted by volunteer coordinating officer. They were given brief information about the study as per "study information sheet for the volunteer through Volunteer coordinating officer" and informed to visit the facility for further process.

Further the volunteers interested in participating in the study were given a detailed explanation on the nature and extent of their participation in the study and the side effects that may occur in simple, non-technical language that suited the individual's level of understanding. All volunteers willing to participate in this study were provided a copy of the consent form describing this study and providing sufficient information for volunteers to make an informed decision regarding their participation in this study. Adequate opportunities were also provided for the volunteers to ask questions and contemplate participation.

The formal consent of a volunteer, using the ethics committee approved consent form, was obtained before the volunteer underwent any study specific procedure. This consent form was signed and dated by the volunteer and by the investigator on 10 Mar 2019. A copy of their signed informed consent document was provided to all subjects.

A copy of 'Study Information Sheet for the volunteer through Volunteer Coordinating Officer', English and Telugu ICFs are provided in Appendix 16.1.3.

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6. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

The following are the key personnel involved in the conduct of the study

Principal Investigator	Dr. P. Venkatesh, MBBS
Sub Investigator	Dr. Sreedhar Reddy Peddi, MD General Medicine, DNB (cardiology)
Clinical Investigator	Dr. S. Ravikishore, MBBS
Study Coordinator	Mr. T. Anil Kumar, M. Pharm
Bioanalytical Investigator	Mr. Praveen Rao M, M. Sc
PK Investigator	Dr. Jaganmohan S, M. Pharm, Ph.D., PDF
Statistical Investigator	Mr. Tushar Shinde, M. Sc
Medical Writer	Mr. Anil Kumar Mandadi, M. Pharm Mr. Y.V.T.Rameswara Reddy, B. Pharm
In-charge - QA & RA	Mr. Madhusudhan K. V, MSc. MBA

A list of CVs of investigators involved in the clinical phase of the study and quality assurance are given in Appendix 16.1.4.

Clinical Facility and Screening Facility	ClinSync Clinical Research Pvt. Ltd., JSR Mall, Plot No.7 to 18, Madinaguda Village, Serilingampally Mandal, Hyderabad-500050, Telangana, India.
Bioanalytical, Pharmacokinetic and Statistical Facility	ClinSync Clinical Research Pvt. Ltd., JSR Mall, Plot No.7 to 18, Madinaguda Village, Serilingampally Mandal, Hyderabad-500050, Telangana, India.
Clinical Laboratory	Medcis Pathlabs India Pvt. Ltd. Plot 16 & 17, Swathi plaza, Bhavani Enclave, Anand Nagar,

	New Bowenpally, Secunderabad-500 011. Phone: 07702 29 29 29 Email: info@medcislabs.com
X ray Facility	ClinSync Clinical Research Pvt. Ltd. Clinical Pharmacology Department, JSR Mall, Plot No.7 to 18, Madinaguda Village, Serilingampally Mandal, Hyderabad-500050, Telangana, India.
Institutional Ethics Committee	Vasavi Institutional ethics committee, Vasavi Medical Research Center #6-1-91, Lakdikapul, Khairatabad, Hyderabad, Telangana 500004 Phone no.: 040-65131333.
Emergency Services	Srikara Hospitals #222&223, Mythri Nagar, Phase-II, Madinaguda, Miyapur, Hyderabad-500049, Ph: 040-47470000, + 91772999 0002.

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7. INTRODUCTION

Patients frequently use more than one medication at a time. Unanticipated, unrecognized, or mismanaged DDIs are an important cause of morbidity and mortality associated with prescription drug use and have occasionally caused the withdrawal of approved drugs from the market. In some instances, understanding how to safely manage a DDI may allow the FDA to approve a drug that would otherwise have an unacceptable level of risk.

The goal of a DDI study with pharmacokinetic endpoints is to inform clinical management strategies by determining whether there is a clinically significant increase or decrease in exposure to the substrate in the presence of the perpetrator. Clinical DDI studies compare substrate concentrations in the absence and presence of a perpetrator drug in vivo.

The relevant concomitant medications for study include those used to treat the same condition for which the investigational drug is being studied or those used to treat common co-morbidities in the patient population.

The purpose of most DDI studies is to determine the ratio of a measure of substrate drug exposure (e.g., AUC ratio) in the presence and absence of a perpetrator drug.

Spironolactone is a specific pharmacologic antagonist of aldosterone indicated for primary hyperaldosteronism, Severe heart failure, Edematous conditions for patients with congestive heart failure, Cirrhosis of the liver, Essential Hypertension, Hypokalemia, and Nephrotic syndrome.

Digoxin is the cardiac (or digitalis) glycoside, having effects on the myocardium and is indicated for the treatment of mild to moderate heart failure, and atrial fibrillation.

Spironolactone and its metabolites interfere with radio immunoassays for digoxin and increase the apparent exposure to digoxin. It is unknown to what extent, if any, spironolactone may increase actual digoxin exposure. In patients taking concomitant digoxin, use an assay that does not interact with spironolactone.

This study was conducted in healthy subjects. The design of this study addresses the primary objective to characterize the pharmacokinetic profile of Digoxin (substrate drug) in the presence and absence of spironolactone (perpetrator) in healthy adult human subjects under fasting condition.

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8. STUDY OBJECTIVES

- **Primary objective**

  To characterize the pharmacokinetic profiles of Digoxin (substrate drug) in the presence and absence of spironolactone (perpetrator) in healthy adult human subjects under fasting condition.

- **Secondary objective**

  To assess whether there is a drug-drug interaction with concomitant use of spironolactone.

  To measure renal clearance (CLR)/Percent Recovered and unchanged drug excreted in urine (fe)/ Amount Recovered for digoxin in the presence and absence of spironolactone (perpetrator) in healthy adult human subjects under fasting condition.

  To monitor safety of the subjects in drug-drug interaction study.

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9. INVESTIGATIONAL PLAN

9.1 OVERALL STUDY DESIGN AND PLAN - DESCRIPTION

Study Design

This study was designed as an open label, balanced, randomized, single-dose, two-treatment, two-sequence, two-period, crossover, drug-drug interaction study in healthy adult human subjects under fasting condition.

Number of Subjects

28 healthy adult male human subjects were enrolled in the study.

Washout period

Washout between treatments: 28 days.

Method of Blinding

This was a randomized open label study. The study personnel involved in the sample analysis were kept blinded from the randomization code during entire study.

Method of Assignment to treatment

The subjects were randomly assigned to one of the possible sequences of treatment A and treatment B as per the randomization scheme generated at ClinSync Clinical Research Pvt. Ltd. according to SAS® (version 9.3).

Either the treatment A or treatment B were administered to 28 subjects in each period of the study.

In both periods (Period-I and II), from Day 1 to Day 5 and Day 7 to Day 9, the subjects who were randomized to treatment B, were administered Spironolactone Oral Suspension 100 mg (Perpetrator; Carospir® Oral Suspension 20 mL of 25 mg / 5 mL), and the subject who were randomized to treatment A were not dosed. On all these days the standard breakfast was served 0.50 hr (30 min) post dose. On Day 6, after overnight fasting of at least 10.00 hours the subjects were dosed either with the treatment A [LANOXIN® (digoxin) USP 250 mcg (substrate drug)] or treatment B [LANOXIN® (digoxin) USP 250 mcg (substrate drug) + Spironolactone Oral Suspension 100 mg (Perpetrator; Carospir® Oral Suspension 20 mL of 25 mg / 5 mL] as per the randomization scheme.

Sequence and Duration of Study Periods

This study was a two treatment, two period, two sequence design. A single oral dose of treatment A and treatment B was administered in period I and period II separated by a wash out period of 28 days between the dosing. The duration of the study from the check in of period I to the last blood sample collection in period II was 39 days.

Housing

Subjects were checked-in to the clinical facility 11.00 hours prior to the drug administration on Day 1 and remain housed in the clinical facility until the collection of 96.00 hours blood sample on Day 10 in each of the study periods. On day 6 the subjects were fasted for at least 10.00 hours prior to dosing.

The protocol and sample case report form have been attached as Appendix 16.1.1 and Appendix 16.1.2, respectively.

9.2 DISCUSSION OF STUDY DESIGN, INCLUDING THE CHOICE OF CONTROL GROUPS

This study was designed as an open label, balanced, randomized, single-dose, two-treatment, two-sequence, two-period, crossover, drug-drug interaction study.

The washout period has been chosen based on the elimination half-life of digoxin, such that at least 5-7 half-lives have elapsed after drug administration. The blood collection time points were chosen to accurately characterize the absorption and elimination phases of the plasma concentration versus time curve obtained after dosing.

9.3 SELECTION OF STUDY POPULATION

A total of 28 healthy adult male human subjects who met all the inclusion and none of the exclusion criteria, were enrolled in the study.

Selection of subjects was done as per the following inclusion and exclusion criteria.

9.3.1 Inclusion Criteria

Volunteer who participated in the study:

a. Healthy human subjects aged between 20 and 35 years (including both)

b. Subjects with a BMI between 18.5 - 24.9 Kg/m^2 (including both) but body weight not less than 60 Kgs.

c. Subjects who were screened at least 48 hours prior to check-in.

d. Subjects with normal health as determined by personal medical history, clinical examination, and laboratory examinations including serological tests during the screening.

e. Subjects with normal 2D echo.

f. Subjects with normal 12-lead electrocardiogram (ECG) or ECG with no clinical significant abnormalities as determined by Investigator.

g. Subjects with normal chest X-Ray (P/A view) or chest X-ray with no clinically significant abnormalities as determined by investigator.

h. Subjects able to communicate effectively.

i. Subjects willing to give written informed consent and adhere to all the

requirements of this protocol.

9.3.2 Exclusion Criteria

Any of the following conditions were cause for exclusion from the study:

a. Subjects having contraindications or hypersensitivity to study drug or related group of drugs.

b. History or presence of any medical condition or disease according to the opinion of the physician.

c. History or presence of significant cardiovascular, pulmonary, hepatic, renal, gastrointestinal, endocrine, immunological, dermatological, neurological or psychiatric disease or disorder.

d. Subject having QT/QTc interval >450 milliseconds.

e. History or presence of significant alcoholism or drug abuse in the past one year.

f. History or presence of significant smoking (more than 10 cigarettes or beedies /day or consumption of tobacco products).

g. Subjects who failed to abstain from consuming any alcoholic products from 48.00 hours prior to check-in to till check-out / last sample of the study.

h. Subjects who failed to abstain from any xanthine-containing food and/or beverages (like chocolate, tea, coffee, cola drinks), cigarettes and tobacco containing products and grapefruit and/or it's juice from 48.00 hours prior to check-in to till check-out / last sample of the study.

i. Subjects who failed to refrain from pan or pan masala, gutkha, masala (containing beetle nut and tobacco) for 48.00 hours prior to check-in to till check-out/last sample of the study.

j. Difficulty with donating blood.

k. Systolic blood pressure less than 110 mm Hg or more than 140 mm Hg.

l. Diastolic blood pressure less than 70 mm Hg or more than 90 mm Hg.

m. Pulse rate less than 60 beats/minute or more than 100 beats/minute.

n. Use of any prescribed medication during last two weeks or OTC medicinal products/ herbal products during the last one week prior to check-in.

o. Major illness during 90 days before check-in.

p. Participation in a drug research study within past 90 days of check-in.

q. Donation of blood (i.e. one unit or 350 mL) in the past 90 days before check-in.

r. Unusual diet consumption in the past 3 weeks before check-in.

9.3.3 Removal of Subjects from Study or Assessment

- If the subject was non-cooperative and non-compliant.
- If the subject found to have entered the study in violation of this protocol.
- If any subject experienced emesis at or before 2 times median T_{max} on Day 6.
- The subject who are suffering from any other clinically significant adverse event.
- The subject who received/required any concomitant medication, which may interfere with the pharmacokinetic property of the study drug.
- The subject who underwent/required hospitalization during the course of the study.
- The subject who reported to the clinical facility for check-in after the prescribed time limit there by failing to meet the protocol requirements.
- If it was felt in the investigator's opinion that it is not in the subject's best interest to continue.
- The subject who was tested positive for Alcohol breath test.
- The subject who was tested positive for any one of the urine drugs of abuse.
- Subjects who missed 03 consecutive blood draws in a study period.

All the subjects were advised that they were free to withdraw from the study at any time. They were also informed that they could be withdrawn from the study by the Principal Investigator/clinical investigator and/ or sponsor in case of un-necessary risk, adverse events or non-compliance.

9.4 TREATMENTS

9.4.1 Treatments Administered

From 11 Mar 2019 (Day 1) to 15 Mar 2019 (Day 5) and from 17 Mar 2019 (Day 7) to 19 Mar 2019 (Day 9) in period I; from 08 Apr 2019 (Day 1) to 12 Apr 2019 (Day 5) and from 14 Apr 2019 (Day 7) to 16 Apr 2019 (Day 9) in period II, the subjects who were randomized to treatment B were administered Spironolactone oral Suspension 100 mg (Perpetrator; Carospir® Oral Suspension 20 mL of 25 mg / 5 mL) and in both period I and II, subjects who were randomized to treatment A were not dosed.

On day 6, after a supervised overnight fasting of 10.00 hours, the subjects were administered a single oral dose of either the Treatment A LANOXIN® (digoxin) USP 250 mcg (substrate drug) or Treatment B LANOXIN® (digoxin) USP 250 mcg (substrate drug) + Spironolactone Oral Suspension 100 mg (Perpetrator; Carospir® Oral Suspension 20 mL of 25 mg / 5 mL) on 16 Mar 2019 in period I and on 13 Apr 2019 in period II, as per the randomization scheme, under fasting condition.

The drug administration details are tabulated in section 9.4.5.

9.4.2 Identity of Investigational Products

Receipt and storage

The Investigational Products along with the Certificates of Analysis (COA) sent by the sponsor were received by the pharmacist. The receipt of the Investigational Products was recorded in the appropriate log. The pharmacist verified that the shipment contains all the items noted in the shipment inventory and also with respect to the COA of the Investigational Products in presence of Quality assurance personnel.

The Investigational Products were stored in locked cabinets in the access controlled pharmacy, which is a secure area, at storage conditions as mentioned in the product label and as per in house SOPs. Access to Investigational Products was provided only to the pharmacist. The Investigational Products were dispensed as per the randomization scheme by the pharmacist in presence of Quality assurance personnel.

The details of the study products are provided in the table below.

Table 6: Identification of Investigational products

Dosage form	Tablet	Suspension
Drug Name	LANOXIN® (digoxin) USP 250 mcg	Carospir® Oral Suspension 20 mL of 25mg / 5 mL (Spironolactone 100 mg)
Manufacturer	--	CMP Development LLC, USA
Manufactured for	Concordia Pharmaceuticals Inc., St. Michael, Barbados BB11005. Initial U.S	--
Lot Number	AH3690B	1118211
Manufacturing Date	18/08/2017	21/11/2018
Expiry Date	07/2021	11/2020

* As per study drug receipt checklist and acknowledgement form

Records of the receipt and dispensing of study products were made to provide complete accountability of the disposition of all supplies. The investigational product accountability details are summarized in table below.

Table 7: Investigational Product Accountability

Particulars	Period	Quantity	Date	Quantity	Date
		Carospir® Oral Suspension 20 mL of 25mg / 5 mL (Spironolactone 100 mg)		**LANOXIN® (digoxin) USP 250 mcg**	
Received		06 bottles (2838 mL)	29 Jan 2019	300 tablets*	29 Jan 2019
		19 bottles (8987 mL)	02 Mar 2019		
Dispensed	Period I	280 ml × 09 days + 20 ml × 09 days #	11 Mar 2019 to 19 Mar 2019	28 + 01#	15 Mar 2019
	Period II	280 ml × 09 days + 20 ml × 09 days #	08 Apr 2019 to 16 Apr 2019	28 + 01#	12 Apr 2019
Dosed	Period I	280 ml × 09 days	11 Mar 2019 to 19 Mar 2019	28	16 Mar 2019
	Period II	280 ml × 09 days	08 Apr 2019 to 16 Apr 2019	28	13 Apr 2019
Remaining Quantity		14 bottles (6425 mL)	--	244	--
Sent to client		Nil	NA	Nil	NA

- 20 mL of Carospir® oral suspension extra dispensed form Day 01 to Day 09 in each period; '01 tablet of LANOXIN® (digoxin) extra dispensed on day 6 in each period.

* - 01 tablet of LANOXIN® (digoxin) were used for physical verification.

Note: 20 mL of Carospir® oral suspension extra dispensed form Day 01 to Day 09 in each period was discarded after dosing under running tap water.

Storage:

The test and the reference products were stored in the pharmacy at appropriate storage conditions.

9.4.3 Method of Assigning Subjects to Treatment Groups

In order to avoid bias, the statistician generated a randomization scheme using statistical analysis software SAS® (Version 9.3). Subjects were randomly assigned to one of the two sequences based on this randomization scheme and was kept under controlled access.

The details of the subjects with the treatments assigned are provided in Appendix 16.1.7.

9.4.4 Selection of Doses in the Study

The Treatment A consisted of a single oral dose of LANOXIN® (digoxin) USP 250 mcg (substrate drug) and the Treatment B consisted of a single oral dose of LANOXIN® (digoxin) USP 250 mcg (substrate drug) + Spironolactone Oral Suspension 100 mg (Perpetrator; Carospir® Oral Suspension 20 mL of 25 mg / 5 mL). The drug-drug interaction (DDI) study using 250 mcg dose with 28 subjects would be useful to establish DDI of digoxin.

9.4.5 Selection and Timing of Dose for each Subject

In each period, from Day 1 to Day 5 and Day 7 to Day 9, the subjects who were randomized B, were administered Spironolactone Oral Suspension 100 mg (Perpetrator; Carospir® Oral Suspension 20 mL of 25 mg / 5 mL) and who were randomized A were not dosed. On these days standard breakfast was served 0.50 hr (30 min) post dose.

On Day 6, the subjects underwent supervised overnight fasting for 10.00 hours before drug administration. Treatment A or Treatment B was administered orally to the subjects according to randomization scheme on Day 6. In case of subjects who were assigned Treatment A, single oral dose of LANOXIN® (digoxin) USP 250 mcg was administered with 240 ± 2 mL of water under fasting condition. Subjects swallowed the tablet as a whole. Dosing compliance was assessed by oral cavity check.

In case of subjects who were assigned Treatment B, single oral dose of LANOXIN® (digoxin) USP 250 mcg was administered with 120 mL of water under fasting condition and Carospir® (spironolactone) Oral Suspension 100 mg (20 mL of 25 mg / 5 mL) was administered with 120 mL of water with a graduated syringe.

CaroSpir (spironolactone) oral suspension administration procedure

20 mL of 25 mg / 5 mL of Carospir® (spironolactone) oral suspension was loaded in the syringes well in advance prior to dosing on the day of dosing. The suspension in the syringe was directly administered to the subjects. Then the syringe was rinsed with approximately 10 mL from 120 mL of dosing water and administered to the subjects. This rinsing procedure was done until the syringe was free of the contents of the medication. Then the remaining quantity of water from the assigned 120 ml water was finally administered to the subjects.

A washout period of 28 days separated the two periods.

On Day 6, subjects who are dosed remained in a supine position or semi recumbent position for 03 hours post-dose and only necessary movement was allowed during this period. Thereafter subject was allowed to ambulate freely during the remaining part of the study. Subject did not sit down (except as directed by the physician secondary to adverse events) during restriction period.

In each period on each day from Day 1 to Day 5 and from Day 7 to Day 9, the subjects were served the standard breakfast, Lunch, Snacks and dinner at 0.50, 4.00, 8.00, and 12.00 hours post dose. On day 6, lunch, snacks and dinner were served at 4.00, 8.00, and 12.00 hours post dose. Meal plans were identical for both the study periods. On Day 6, water was provided ad libitum during the study, except 1 hour before and 1 hour after dosing except during drug administration.

Drug administration details are provided in the table below.

Table 8: Drug Administration

Period Number	Subject Number		Drug Administration	
	Treatment A	Treatment B	Date	Time
One	--	01, 15	11 Mar 2019 to 19 Mar 2019	08:30
	02, 16	--		08:32
	17	03		08:34
	04	18		08:36
	19	05		08:38
	06	20		08:40
	21	07		08:42
	08	22		08:44
	--	09, 23		08:46
	10, 24	--		08:48
	25	11		08:50
	12	26		08:52
	13	27		08:54
	28	14		08:56
Two	01, 15	--	08 Apr 2019 to 16 Apr 2019	08:30
	--	02, 16		08:32
	03	17		08:34
	18	04		08:36
	05	19		08:38
	20	06		08:40
	07	21		08:42
	22	08		08:44
	09, 23	--		08:46
	--	10, 24		08:48
	11	25		08:50
	26	12		08:52
	27	13		08:54
	14	28		08:56

Treatment A: LANOXIN® (digoxin) USP 250 mcg

Treatment B: LANOXIN® (digoxin) USP 250 mcg + Carospir® (spironolactone) oral suspension 100 mg (20 mL of 25 mg / 5 mL)

9.4.6 Blinding

This study was designed as an open label study. However, the bioanalytical scientists who performed the analysis were blinded to the treatment during subject sample analysis to avoid bias.

9.4.7 Prior and Concomitant Therapy

No concomitant medication was administered since there were no AEs recorded in the study.

9.4.8 Treatment Compliance

In each period, from Day 1 to Day 5 and Day 7 to Day 9, the subjects who were assigned B, received LANOXIN® (digoxin) USP 250 mcg + Spironolactone Oral Suspension 100 mg (Carospir® Oral Suspension 20 mL of 25 mg / 5 mL) and subjects who were assigned A were not dosed. All the subjects swallowed the drug as a whole. On Day 6, subjects received a single dose of the Treatment A or Treatment B in each period. All the subjects swallowed the drug as a whole.

The Principal investigator or qualified designate, was responsible for ensuring that the administration of timed oral doses was conducted. Additionally, to ensure subject compliance, study personnel conducted oral cavity check immediately after drug administration.

9.5 EFFICACY AND SAFETY VARIABLES

9.5.1 Pharmacokinetic and Safety Measurements Assessed

Pharmacokinetic measurements assessed:

The following pharmacokinetic parameters for digoxin were obtained using non-compartmental method (WinNonlin, version 8.1, Pharsight Corporation, USA): C_{max}, AUC_{0-96} and t_{max}

Safety Assessment:

Subjects were monitored for safety during the study and until the completion of the study. Safety assessments were done based on clinical observations, laboratory data at the beginning and at the end of the study and evaluation of the AEs observed during the course of the study.

Screening assessment comprised of detailed demographic data and medical history followed by general physical examination and systemic examination and laboratory investigations (hematology, biochemistry, urine analysis and serology), vitals, ECG

(done within 28 days prior to check in of period one), 2D echo and chest X-ray (done within 6 months prior to check in of period one).

Subjects were monitored for safety during the entire study. The subject's Clinical examination along with vital signs (seated blood pressure, radial pulse rate and oral/aural temperature) were measured during check-in and check-out of each period.

Clinical examination was done on prior to dosing on Day 06 in each study period. Oral/aural temperature was measured prior to dosing in each study period.

Vital parameters – seated blood pressure and radial pulse rate were measured at prior to dosing, 1.00, 3.00, 5.00 and 11.00 hours post dose in each period from Day 1 to Day 5, and from Day 6 to Day 9, vitals were measured prior to dosing, at 1.00, 2.00, 3.00, 5.00, 8.00 and 11.00 hours post dose in each period.

Note

- On Day 6 the Vital signs (i.e. up to 3.00 hours post dose) was performed at bed side.
- For subjects who were randomized to treatment A, the vitals were checked at 5.00 hours post dose during day 1 to Day 5.

ECG was performed at 1.00 and 3.00 hours post dose in each period on Day 6, before period II check-in for subject study eligibility and at check-out of each period of the study.

In each study period creatinine clearance and serum potassium levels were measured on Day 05 for the dosing eligibility of the subjects.

For the safety of the subjects, hematology (except blood grouping and Rh typing) and biochemistry (except random blood sugar) investigations were repeated at the end of the study.

9.5.2 Appropriateness of Measurements

The blood sampling points were chosen such that T_{max} could be accurately characterized for digoxin. In addition, sampling was done up to 96.00 hours post dose such that the plasma concentration could be measured for adequately profiling the pharmacokinetics of the product.

All safety assessments chosen were standard and widely used and are documented in the appropriate section of the case report forms (CRFs).

9.5.3 Primary Pharmacokinetic Variable(s)

The primary pharmacokinetic variables calculated in the study were C_{max} and AUC_{0-96} for digoxin. The absence of drug-drug interaction was based on the 90% Confidence Intervals (CI) of the above parameters.

9.5.4 Drug Concentration Measurements

9.5.4.1 Blood Sampling

On Day 6, a total of 25 (1 x 5 mL) blood samples were collected for each subject in pre-specified vacutainer tubes containing anticoagulant (K_2EDTA) in each study period. The pre-dose sample was collected within 2 hours prior to drug administration. The post-dose blood samples were collected at 0.25, 0.50, 0.75, 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00, 36.00, 48.00, 60.00, 72.00 and 96.00 hours in each study period.

Note: Blood samples up to 3 hours post dose were collected at bed side.

The pre-dose and post-dose samples up to 24.00 hours were collected via an indwelling cannula placed in an ante-cubital vein or one of the forearm veins inserted prior to first sample collection in each period and 36.00, 48.00, 60.00, 72.00 and 96.00 post dose samples were collected by a fresh vein-puncture.

All the blood draws up to 96.00 hours were done in-house. The collected blood was transferred immediately after collection in vacutainer tubes containing anticoagulant (K_2EDTA) in each of the two study periods.

Sample Processing:

The vacutainers containing blood samples obtained from the subjects were centrifuged in a refrigerated centrifuge at 4000 rpm for 10 minutes at 4°C. The separated plasma was transferred to pre-labeled polypropylene tubes to make equal aliquots, for all the samples.

Blood volume:

The total volume of blood collected during the entire study did not exceed 292 mL for each subject. However, additional blood (3-5 mL) would be collected for repeat/ additional investigations when significant abnormalities in the laboratory investigations were observed.

Sample Storage and Shipping:

All plasma samples were stored in a freezer at a temperature of -20 ± 5°C up to 4.00 hours and then were transferred to -70 ± 15°C at the clinical site. Prior to shipping, the samples were packed into thermal insulated containers and packed in sufficient dry ice to assure that they remained frozen and were protected from breakage during shipment. The plasma samples were transferred from cold chain management (CCM) department to bioanalytical facility on 23 Apr 2019 for analysis batch wise as per approved sequence schedule, after the completion of clinical phase.

9.5.4.2 Urine Sampling

On Day 6, a total of 11 urine samples were collected from each subject in each study period at intervals of (0.00-1.00), (1.00-2.00), (2.00-4.00), (4.00-6.00), (6.00-8.00),

(8.00-12.00), (12.00-16.00), (16.00-24.00), (24.00-48.00), (48.00-72.00) and (72.00-96.00) hours post dose.

Immediately out of the total volume of urine collected at each collection time interval, approximately 10 mL (2 x 5 mL aliquots) of urine samples were transferred into two pre-labeled containers for analysis. They were stored at deep freezer maintained at-20°C or lower until dispatched from Clinical Unit to Bioanalytical Department on 24 May 2019 for analysis. The analysis was done after the completion of clinical phase.

The urine samples were stored at -20°C or lower in the Bioanalytical facility until analysis. After collecting the urine samples from all the subjects at each collection time interval, the total pooled volume was recorded.

9.5.4.3 Method of Measurement

The plasma and urine samples were shipped to the bioanalytical facility of ClinSync Clinical Research Pvt Ltd, Hyderabad, India, where the samples were analyzed to determine the drug concentrations. The drug concentrations were determined from all the plasma samples received from the clinical phase using a validated LC-MS/MS method.

9.6 DATA QUALITY ASSURANCE

A systematic and independent examination of study activities and documents was conducted to determine whether the activities were conducted, and the data were recorded, analyzed, and accurately reported according to the protocol, Standard Operating Procedures (SOPs), Good Clinical Practice (GCP), Good Laboratory Practice (GLP) and other applicable regulatory requirement(s).

The quality assurance unit audited every phase of the study from enrollment of subjects into the study to the accurate and completeness of the final reports. The QA statement/declaration is given in Appendix 16.1.8.

9.7 STATISTICAL METHODS PLANNED IN THE PROTOCOL AND DETERMINATION OF SAMPLE SIZE

9.7.1 Statistical and Analytical Plans

9.7.1.1 Pharmacokinetic Analysis

Pharmacokinetic parameters for digoxin was calculated from the plasma concentration data and urine data were obtained from all the subjects who completed both the periods, using standard, non-compartmental methods with WinNonlin® Software (version 8.1, Pharsight Corporation, USA). The definition and method of determination for each pharmacokinetic variable is summarized as follows:

Primary Pharmacokinetic parameters in plasma

C_{max}	Maximum measured plasma concentration.
AUC_{0-96}	Area under the plasma concentration versus time curve from time 0 to the 96 hour time point concentration.

Secondary Pharmacokinetic parameters

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

Pharmacokinetic parameters in urine

CL_R	Renal clearance $Ae_{0-240h}/AUC_{0-240\ h}$
f_e	Unchanged drug excreted in urine

Concentration-time data that were below limit of quantification were treated as zero for calculations and summary statistics.

Pharmacokinetic analyses included data from samples of subjects who completed both periods of the study.

Descriptive statistics for concentration data were presented for scheduled (nominal) sampling times. Actual elapsed times were used for all pharmacokinetic and statistical analyses only if the samples were withdrawn outside of the permitted window period.

Samples lost / not received from the clinical site, samples processing or arising due to drop out or withdrawal of subjects were to be denoted by the character "M" and excluded from pharmacokinetic analysis.

9.7.1.2 Statistical Analysis

Statistical analysis was performed using the SAS® software version 9.3 (SAS Institute Inc., Cary NC, USA) for digoxin. The following summary statistics for the pharmacokinetic parameters were calculated for both the Treatment A and Treatment B: Number of Subjects (N), Arithmetic Mean (Mean), Standard Deviation (SD), Minimum, Maximum, Median and Percentage Coefficient of Variation (CV%). Additionally, Geometric Mean (GM) was estimated for C_{max} and AUC_{0-96}.

The log transformed pharmacokinetic parameters (C_{max} and AUC_{0-96} for digoxin) were analyzed using Type III sum of squares, with the main effects of formulation, period, sequence and subjects nested within sequence as random effect. The 90% confidence intervals for the difference between drug formulation least-square means

(LSM) were calculated for the parameters C_{max} and AUC_{0-96} using log transformed data.

9.7.2 Determination of Sample Size

A total of 28 healthy human adult subjects were included in the study. Additionally, 04 stand-by subjects were included in order to dose 28 subjects in period I. As the same perpetrator (LANOXIN® Tablets USP 250 mcg) would be given to both the treatments and only the difference could be with and without spironolactone oral suspension, 28 subjects along with 04 stand-by subjects would be sufficient to provide a reliable estimate of the magnitude and variability of the interaction.

9.8 CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

There were no changes in the clinical conduct of the study or planned analyses. The study was conducted as per the IEC approved Protocol.

10. STUDY SUBJECTS

10.1 DISPOSITION OF SUBJECTS

A total of 28 + 04 (standby subjects) healthy adult human male subjects were enrolled in the study. All these 28 subjects were dosed in Period-I and Period-II of the study.

The following table summarizes the subject disposition:

Table 9: Subject disposition (All Subjects)

	Sequence 1 AB	Sequence 2 BA	All Subjects
Subjects			
Randomized	14	14	28
Completed	14	14	28
Discontinued	00	00	00

Treatment A: LANOXIN® (digoxin) USP 250 mcg (substrate drug)

Treatment B: LANOXIN® (digoxin) USP 250 mcg + Spironolactone Oral Suspension 100 mg (Carospir® Oral Suspension 20 mL of 25 mg / 5 mL)

10.2 PROTOCOL DEVIATIONS

Protocol Deviations occurred during the entire duration of the study was presented in the Appendix 16.2.2.

Sample Centrifuge Deviation

The 0.25 hr sample in period I was centrifuged 45 minutes beyond the scheduled time of blood sample collection

Blood Sample Collection Deviations

There were three sample collection deviations reported in this study.

11.00 hours post dose vitals Deviations

There were seven post dose vitals deviations were recorded in this study.

11. PHARMACOKINETIC EVALUATION

11.1 DATA SETS ANALYSED

A total of 28 subjects were dosed and randomized. All these 28 subjects had evaluable pharmacokinetic parameters for both the periods 1 and 2 and were included in Pharmacokinetic analysis.

Pharmacokinetic analysis was performed on plasma concentration data and urine concentration data from subject nos. 01 - 28.

11.2 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

A total of 28 subjects were enrolled in this study. All subjects were healthy human adult male subjects of South Asian race (Indian). Their mean (±SD) age was 28.86 ± 3.51 years (range 20 to 35), mean (±SD) height, weight and BMI were 169.21 ± 5.79 cms (range 156 to 180), 66.50 ± 4.11 kg (range 60.50 to 74.20) and 23.27 ± 1.68 kg/m^2 (range 19.61 to 24.86) respectively.

A table detailing the subject wise demographic characteristics is given in Appendix 16.2.4.

11.3 MEASUREMENTS OF TREATMENTS COMPLIANCE

Treatment compliance was measured by analysis of plasma and urine samples to determine the concentration of digoxin following the administration of Treatment A and Treatment B.

The assessment of treatment compliance was performed as given under section 9.4.8. The analysis of the clinical samples was performed as explained under section 9.5.4. The details of the validated methods are provided in separate method validation report given in Appendix 16.5.

The samples were analyzed using the above validated method.

The quality control samples were prepared and distributed between subject samples in each analytical run both in the presence and absence of spiranolactone and its metabolite canrenone.

Quality Control samples were analyzed with samples in order to monitor the accuracy and precision of the validated method to ensure that the method continues to perform satisfactorily. The details of the subject analysis are provided in a separate bioanalytical report given in Appendix 16.5.

11.4 PHARMACOKINETIC RESULTS AND TABULATIONS OF INDIVIDUAL SUBJECT DATA

11.4.1 Pharmacokinetic & Statistical Analysis

Pharmacokinetic analysis of the plasma and urine concentrations for digoxin was carried out for Treatment A and Treatment B to determine the following PK parameters in each individual subject using Phoenix WinNonlin version 8.1. The results are given below:

Concentration-time data that are below limit of quantification were treated as zero for calculations and summary statistics.

Summary statistics for concentration data were presented for scheduled (nominal) sampling times while actual elapsed times were used for all pharmacokinetic and statistical analyses unless the samples were withdrawn within the permitted window period.

The mean concentration-time profiles of digoxin in plasma after administration of Treatment A and Treatment B are presented in Figure 1 and Figure 2 respectively. Summary statistics of PK parameters per treatment for digoxin are shown in Table 10.

Summary statistics of pharmacokinetic profile of digoxin (substrate drug) in the presence and absence of spironolactone (perpetrator) was provided based on plasma and urine data. Renal clearance (CLR)/Percent Recovered and unchanged drug excreted in urine (fe)/ Amount Recovered for digoxin was measured and provided as a supporting data (evidence) in Table 12.

All the subjects included in the pharmacokinetic analysis had sufficient quantifiable concentrations versus time and no exclusions were considered with respect to pharmacokinetic analysis.

Figure 1 Mean Plasma concentration vs time profiles - treatment wise of digoxin (Linear graph)

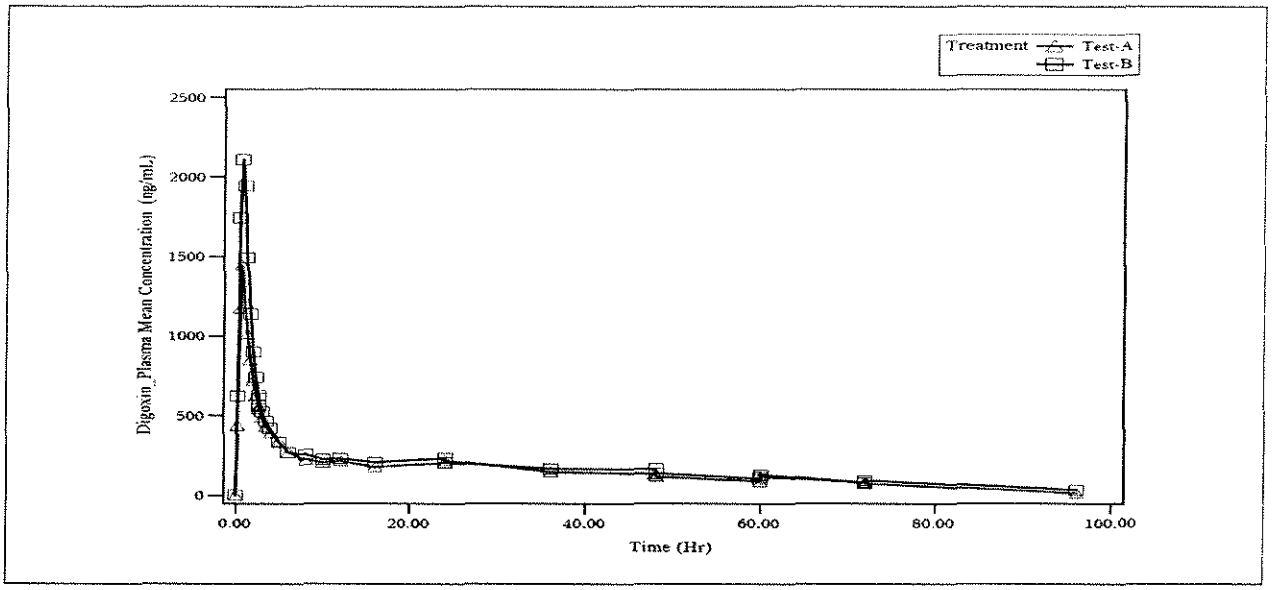

Figure 2 Mean Plasma concentration vs time profiles - treatment wise of digoxin (Semi-log graph)

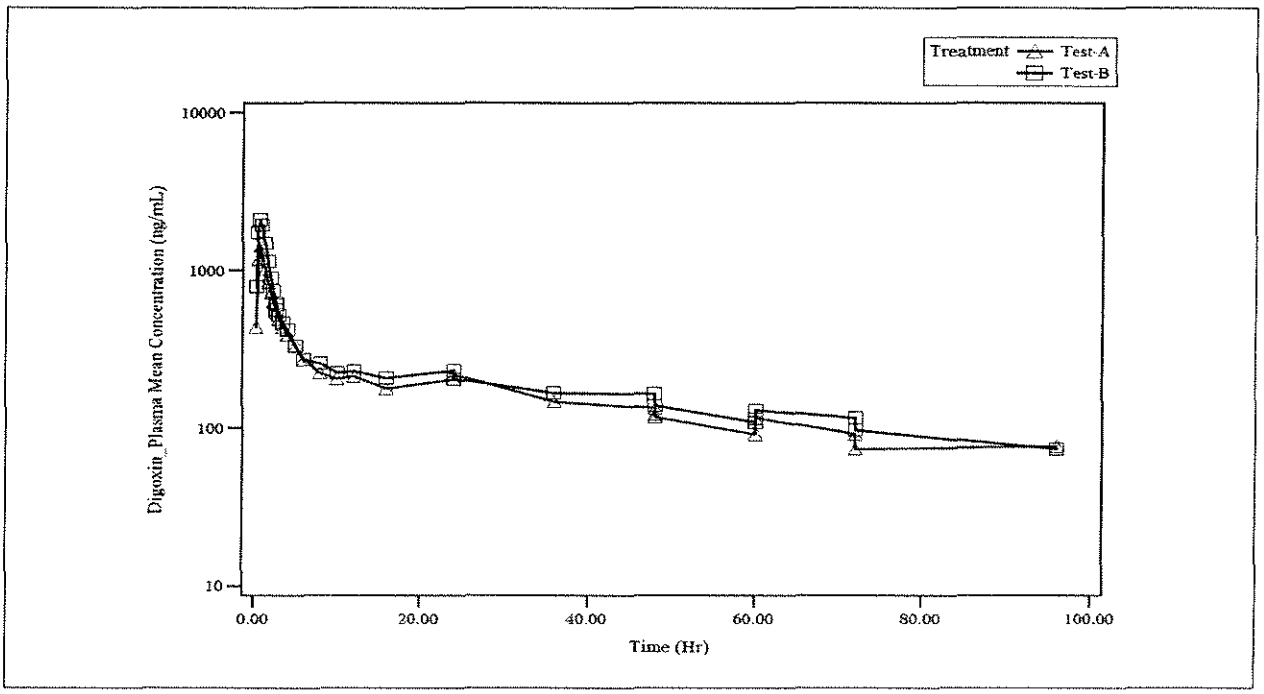

Table 10: Summary Statistics for Untransformed Plasma PK Parameters of digoxin Per Treatment

Treatment	Statistics	C_{max} (ng/mL)	AUC_{0-96} (ng.hr/mL)	T_{max} (h)
Treatment A	N	28	28	28
	Mean	1530.188	13715.974	0.744
	SD	502.810	2669.003	0.195
	Min	644.613	8632.172	0.500
	Median	1429.822	13440.345	0.750
	Max	2734.608	21455.761	1.330
	Geometric Mean	1456.085	13473.043	0.721
	CV % Geometric Mean	32.993	19.431	25.996
Treatment	**Statistics**	**C_{max} (ng/mL)**	**AUC_{0-96} (ng.hr/mL)**	**T_{max} (h)**
Treatment B	N	28	28	28
	Mean	2432.908	16551.500	0.901
	SD	870.562	4108.500	0.569
	Min	539.707	6373.657	0.500
	Median	2422.385	17151.091	0.750
	Max	5044.130	23997.624	3.500
	G. Mean	2254.118	15996.644	0.810
	G.mean %CV	45.789	28.453	44.310

Treatment A: LANOXIN® (digoxin) USP 250 mcg (substrate drug)

Treatment B: LANOXIN® (digoxin) USP 250 mcg + Spironolactone Oral Suspension 100 mg (Carospir® Oral Suspension 20 mL of 25 mg / 5 mL)

The median time to reach peak plasma concentration (T_{max}) for digoxin was 0.750 hr and 0.750 hr following administration of Treatment A and Treatment B respectively. The T_{max} ranged from 0.500 to 1.330 hr and 0.500 to 3.500 hr for Treatment A and Treatment B respectively.

The exposure (C_{max} and AUC_{0-96}) of digoxin was not comparable for the Treatment A and Treatment B. The geometric mean C_{max} was 1456.085 and 2254.118 ng/mL for Treatment A and Treatment B respectively. The geometric mean AUC_{0-96} was 13473.043 and 15996.644 ng.hr/mL for Treatment A and Treatment B respectively.

Drug-Drug Interaction (DDI)

Statistical analysis was performed to assess the DDI as mentioned in Section 9.7.1. Geometric means, Geometric mean ratio and 90% Confidence intervals for Treatment A and Treatment B are provided in Table 9. The 90% confidence intervals of the geometric mean ratio (Treatment A / Treatment B) for C_{max} and AUC_{0-96} were not within standard BE limits.

Table 11 Summary of Statistical Analysis of Ln-transformed Pharmacokinetic Parameters of digoxin

PK Parameters (Unit)	Geometric Least Square Means and It's Ratio			ISCV (%)	90% CI	Power (%)
	Treatment A	Treatment B	(Treatment A / Treatment B) (%)			
C_{max} (ng/mL)	1456.085	2254.118	64.597	40.07	54.18% to 77.02%	67.47
AUC_{0-96} (ng.hr/mL)	13473.043	15996.644	84.224	18.56	77.45% to 91.59%	99.56

Treatment A: LANOXIN® (digoxin) USP 250 mcg (substrate drug)

Treatment B: LANOXIN® (digoxin) USP 250 mcg + Spironolactone Oral Suspension 100 mg (Carospir® Oral Suspension 20 mL of 25 mg / 5 mL)

C_{max} and AUC_{0-96}:

The Treatment A was compared to the Treatment B with respect to the pharmacokinetic variables C_{max} and AUC_{0-96} for digoxin using an analysis of variance with Treatment, period, sequence and subject nested within sequence effects after a logarithmic transformation of the data. Point estimates and 90% confidence intervals for the "Treatment A / Treatment B" mean ratios of these variables were calculated.

Table 12 Summary Statistics for Untransformed Urine PK Parameters of digoxin Per Treatment

Treatment	Statistics	Renal clearance (CLR)/Percent Recovered	unchanged drug excreted in urine (fe)/ Amount Recovered
Treatment A	N	28	28
	Mean	54.687	136717.72
	SD	29.158	72895.492
	Min	22.180	55449.800
	Median	46.111	115277.98
	Max	147.642	369104.30
	G. Mean	49.042	122606.02
	G.mean %CV	48.257	48.257

Treatment	Statistics	Renal clearance (CLR)/Percent Recovered	unchanged drug excreted in urine (fe)/ Amount Recovered
Treatment B	N	28	28
	Mean	53.378	133446.07
	SD	21.256	53141.044
	Min	21.641	54103.200
	Median	51.185	127962.08
	Max	95.602	239003.90
	G. Mean	49.410	123523.87
	G.mean %CV	42.201	42.201

Treatment A: LANOXIN® (digoxin) USP 250 mcg (substrate drug)

Treatment B: LANOXIN® (digoxin) USP 250 mcg + Spironolactone Oral Suspension 100 mg (Carospir® Oral Suspension 20 mL of 25 mg / 5 mL)

The Renal clearance (CLR)/Percent Recovered and unchanged drug excreted in urine (fe)/ Amount Recovered of digoxin was comparable for the Treatment A and Treatment B. The geometric mean Renal clearance (CLR)/Percent Recovered was 49.042 and 49.410 for Treatment A and Treatment B respectively. The geometric mean unchanged drug excreted in urine (fe)/ Amount Recovered was 122606.02 and 123523.87 for Treatment A and Treatment B respectively.

11.4.2 Statistical / Analysis Issues

Not applicable

11.4.2.1 Adjustments for Covariates

There were no covariate adjustments made during statistical analysis.

11.4.2.2 Handling of Dropouts or Missing Data

Pharmacokinetic analyses included data from samples of subjects who completed both study periods.

The 90% confidence intervals are based on those subjects who completed both the periods of the study.

11.4.2.3 Interim Analyses and Data Monitoring

Not applicable.

11.4.2.4 Multicentre Studies

This was a single center study.

11.4.2.5 Multiple Comparison/Multiplicity

Not applicable

11.4.2.6 Use of an "Efficacy Subset" of Subjects

Not applicable

11.4.2.7 Active- Control Studies Intended to Show Equivalence

Not applicable

11.4.2.8 Examination of Subgroups

Not applicable

11.4.3 Tabulation of Individual Response Data

The individual concentration data of digoxin was provided in Appendix 16.2.6.

11.4.4 Drug Dose, Drug Concentration, and Relationships to Response

Not applicable

11.4.5 Drug-Drug and Drug-Disease Interactions

Not applicable

11.4.6 By – Subject Displays

Individual plasma Concentrations Versus Time (Linear plots) for digoxin was provided in Appendix 16.2.6.

11.4.7 Pharmacokinetic Conclusions

The % Treatment A / Treatment B ratio of digoxin was 64.597% and 84.224% for C_{max} and AUC_{0-96} respectively. The lower and the upper limits at 90% confidence interval were 54.18% - 77.02% and 77.45% - 91.59% for C_{max} and AUC_{0-96} respectively, which is not within the standard equivalence limit of 80.00-125.00%. Based on the above results presence of drug-drug interaction is established between digoxin and spironolactone.

By comparing C_{max} and AUC_{0-96} plasma values of Treatment A and Treatment B, we conclude that these values were increased by 35% and 16% respectively for Treatment B

The Renal clearance (CLR)/Percent Recovered and unchanged drug excreted in urine (fe)/ Amount Recovered of digoxin was comparable for the Treatment A and Treatment B. The geometric mean Renal clearance (CLR)/Percent Recovered was 49.042 and 49.410 for Treatment A and Treatment B respectively. The geometric mean unchanged drug excreted in urine (fe)/ Amount Recovered was 122606.02 and 123523.87 for Treatment A and Treatment B respectively.

By comparing these values, we conclude that there is not much difference between Treatment A and Treatment B, with respect to Renal clearance (CLR)/Percent Recovered and unchanged drug excreted in urine (fe)/ Amount Recovered.

12. SAFETY EVALUATION

12.1 EXTENT OF EXPOSURE

On Day 6, subjects were on fast for 10.00 hours prior to dose administration and for 4.00 hours post dose in each study period. Subjects were administered a single dose of either Treatment A or Treatment B as per randomization scheme with about 240 mL of water for the subjects who were assigned A; 120 mL for LANOXIN® (digoxin) USP 250 mcg and 120 mL for Spironolactone Oral Suspension 100 mg (Carospir® Oral Suspension 20 mL of 25 mg / 5 mL) for the subjects who were assigned B, at ambient temperature in sitting position in each study period. Oral cavity check was done immediately after drug administration to assess the compliance to this procedure.

Total 28 subjects had completed the clinical phase of the study successfully. Plasma and urine samples of 28 subjects were analyzed and plasma and urine concentration data of all 28 subjects was considered to draw statistical conclusion.

The duration of clinical phase was 39 days, including the washout period of 28 days between each study period.

12.2 ADVERSE EVENTS (AEs)

12.2.1 Brief summary of adverse events

There were no adverse events reported in this study.

12.2.2 Display of adverse events

There were no adverse events reported in this study.

12.2.3 Analysis of Adverse Events

There were no adverse events reported in this study.

12.2.4 Listing of Adverse Events by Subject

There were no adverse events reported in this study.

12.3 DEATHS, OTHER SERIOUS ADVERSE EVENTS, AND OTHER SIGNIFICANT ADVERSE EVENTS

There were no deaths or serious adverse events reported in this study.

12.3.1 Listing of Deaths, other Serious Adverse Events and Other Significant Adverse Events

Not applicable

12.3.1.1 Deaths

Not applicable

12.3.1.2 Other Serious Adverse Events

Not applicable

12.3.1.3 Other Significant Adverse Events

Not applicable

12.3.2 Narratives of Deaths, Other Serious Adverse Events and Certain Other Significant Adverse Events

Not applicable

12.3.3 Analysis and Discussion of Deaths, Other Serious Adverse Events and Other Significant Adverse Events

Not applicable

12.4 CLINICAL LABORATORY EVALUATION

12.4.1 Listing of Individual Laboratory Measurements by Subject

The individual laboratory measurements of each subject were given in Appendix 16.2.8.

12.4.2 Evaluation of Laboratory Parameter

12.4.2.1 Laboratory Values over Time

The screening clinical laboratory investigations included hematology, serum biochemistry, serology and urine analysis as detailed in the protocol. The hematology (except blood grouping and Rh typing) and biochemistry (except random blood sugar) investigations done at screening were repeated at the end of the study.

12.4.2.2 Individual Subject Change

Not applicable

12.4.2.3 Individual Clinically Significant Abnormalities

For individual clinically non significant abnormalities refer to Table 16 in section 14.3.4.

12.5 VITAL SIGNS, PHYSICAL FINDINGS AND OTHER OBSERVATIONS RELATED TO SAFETY

Subjects were monitored for safety and tolerability during the entire study. The subject's Clinical examination along with vital signs (seated blood pressure, radial pulse rate and oral/aural temperature) were measured during check-in and check-out of each period.

Clinical examination was done on prior to dosing on Day 06 in each study period.

Oral/aural temperature was measured prior to dosing in each study period.

Vital parameters – seated blood pressure and radial pulse rate were measured at prior to dosing, 1.00, 3.00, 5.00 and 11.00 hours post dose in each period from Day 1 to Day 5, and from Day 6 to Day 9, vitals were measured prior to dosing, at 1.00, 2.00, 3.00, 5.00, 8.00 and 11.00 hours post dose in each period.

Note

- On Day 6 the Vital signs (i.e. up to 3.00 hours post dose) was performed at bed side.
- From day 1 to Day 5, subjects who were randomized to treatment A, the vitals were checked at 5.00 hours post dose.

ECG was performed at 1.00 and 3.00 hours post dose in each period on Day 6, before period II check-in for subject study eligibility and at check-out of each period of the study. 2D echo was performed at the time of screening.

In each study period creatinine clearance and serum potassium levels were measured on Day 05 for the dosing eligibility of the subjects.

The screening clinical laboratory investigations included hematology, serum biochemistry, serology and urine analysis as detailed in the protocol. The hematology (except blood group and Rh typing) and biochemistry (except random blood sugar) investigations done at screening were repeated at the end of the study.

12.6 SAFETY CONCLUSIONS

There were no adverse events reported in this study.

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13. DISCUSSION AND OVERALL CONCLUSIONS

This was an open label, balanced, randomized, single-dose, two-treatment, two-sequence, two-period, crossover, drug-drug interaction study in healthy adult human subjects under fasting condition.

This study was conducted in compliance with the final protocol, the applicable International Conference on Harmonization Guidelines for Good Clinical Practice (GCP), the relevant sections of Good Laboratory Practice (GLP), local laws and regulations (ICMR Guidelines on Biomedical Research, Schedule Y (amended version, 2014) of CDSCO (Central Drugs Standard Control Organization), relevant sections of Drugs and Cosmetics (First Amendment) Rules 2013, CDSCO Bioavailability & Bioequivalence guidelines, applicable USFDA guidelines, the provisions of Declaration of Helsinki (Brazil, October 2013) and applicable in- house SOP's.

The primary objective of this study was to characterize the pharmacokinetic profile of Digoxin (substrate drug) in the presence and absence of spironolactone (perpetrator) in healthy adult human subjects under fasting condition.

The secondary objective of this study was

- To assess whether there is a drug-drug interaction with concomitant use of spironolactone.
- To measure renal clearance (CLR)/Percent Recovered and unchanged drug excreted in urine (fe)/ Amount Recovered for digoxin in the presence and absence of spironolactone (perpetrator) in healthy adult human subjects under fasting condition
- To monitor safety of the subjects in drug-drug interaction study

The clinical part of the study was conducted in 28 subjects and the plasma samples and urine samples were sent to Bioanalytical department, ClinSync Clinical Research Pvt. Ltd., Hyderabad, where the bioanalysis was performed using validated LCMS/MS method. The details have been presented in a separate Bioanalytical Report. The pharmacokinetic parameters C_{max}, AUC_{0-96}, and T_{max} for digoxin in plasma, Renal clearance (CLR) /Percent Recovered and Unchanged drug excreted in urine (fe)/Amount Recovered in urine were obtained using non-compartmental analysis (WinNonlin, version 8.1, Pharsight Corporation, USA). All statistical analyses were performed using SAS® version 9.3 (SAS Institute Inc., Cary, NC, USA).

Sampling was done up to 96.00 hours such that the plasma concentration and urine samples could be measured for adequately profiling the pharmacokinetics of the product and study periods were separated by a washout period of 28 days for

complete elimination of the product substance. The pharmacokinetic and statistical analysis was done on all the 28 subjects.

The median time to reach peak plasma concentration (T_{max}) for digoxin was 0.750 hr and 0.750 hr following administration of Treatment A and Treatment B respectively. The T_{max} ranged from 0.500 to 1.330 hr and 0.500 to 3.500 hr for Treatment A and Treatment B respectively.

The exposure (C_{max} and AUC_{0-96}) of digoxin was not comparable for the Treatment A and Treatment B. The geometric mean C_{max} was 1456.085 and 2254.118 ng/mL for Treatment A and Treatment B respectively. The geometric mean AUC_{0-96} was 13473.043 and 15996.644 ng.hr/mL for Treatment A and Treatment B respectively.

The intra-subject variability of digoxin for C_{max} and AUC_{0-96} was 40.07% and 18.56% respectively.

Absence of drug-drug interaction would be established if the 90% confidence interval for the ratio of population geometric means between two treatments (A and B), based on log-transformed data, present in the equivalence limits of 80.00 - 125.00% for C_{max} and AUC_{0-96}.

The % Treatment A / Treatment B ratio of digoxin was 64.597% and 84.224% for C_{max} and AUC_{0-96} respectively. The lower and the upper limits at 90% confidence interval were 54.18% - 77.02% and 77.45% - 91.59% for C_{max} and AUC_{0-96} respectively, which is not within the standard equivalence limit of 80.00-125.00%. Based on the above results presence of drug-drug interaction is established between digoxin and spironolactone.

By comparing C_{max} and AUC_{0-96} plasma values of Treatment A and Treatment B, we conclude that these values were increased by 35% and 16% respectively for Treatment B.

The Renal clearance (CLR)/Percent Recovered and unchanged drug excreted in urine (fe)/ Amount Recovered of digoxin was comparable for the Treatment A and Treatment B. The geometric mean Renal clearance (CLR)/Percent Recovered was 49.042 and 49.410 for Treatment A and Treatment B respectively. The geometric mean unchanged drug excreted in urine (fe)/ Amount Recovered was 122606.02 and 123523.87 for Treatment A and Treatment B respectively.

By comparing these values, we conclude that there is not much difference between Treatment A and Treatment B, with respect to Renal clearance (CLR)/Percent Recovered and unchanged drug excreted in urine (fe)/ Amount Recovered.

14. TABLES, FIGURES AND GRAPHS REFERRED TO BUT NOT INCLUDED IN THE TEXT

14.1 DEMOGRAPHIC DATA

Table 13: Summary Data of subjects enrolled and considered to establish DDI

	N = 28 (subjects enrolled and considered for the equivalence)			
	Age (yrs)	**Height (cms)**	**Weight (kg)**	**BMI (kg/m^2)**
Mean	28.86	169.21	66.50	23.27
SD	3.51	5.79	4.11	1.68
CV%	12.18	3.42	6.18	7.21
Minimum	20	156	60.50	19.61
Maximum	35	180	74.20	24.86

14.2 PHARMACOKINETIC DATA

Table 14: Plasma Pharmacokinetic Parameters of digoxin for Treatment A

Subject	Period	C_{max} (ng/ml)	AUC_{0-96} (hr)*(ng/ml)	T_{max} (hr)
1	2	1231.789	12257.410	0.75
2	1	1632.702	12534.276	1.00
3	2	1119.346	12320.487	0.50
4	1	1555.440	14817.567	0.75
5	2	1200.204	8632.172	0.50
6	1	1337.122	15543.572	0.50
7	2	2734.608	15261.849	0.50
8	1	1096.518	13897.911	1.00
9	2	1508.190	12497.649	0.75
10	1	1432.604	16663.802	0.75
11	2	1259.124	12280.198	0.50
12	1	1642.628	12456.512	0.75
13	1	1653.681	14957.186	0.75
14	2	1347.203	13449.781	1.33
15	2	1345.378	14351.545	0.75
16	1	1427.040	12256.029	0.75
17	1	1326.221	14119.301	0.75
18	2	2520.483	12229.820	0.75
19	1	2174.565	13430.909	0.75
20	2	2673.194	17461.106	0.50
21	1	1135.270	15705.494	1.00
22	2	1664.995	10134.973	0.50
23	2	1043.906	15889.401	0.75
24	1	1593.511	11630.262	0.75
25	1	862.546	17061.128	0.75
26	2	644.613	9969.226	1.00
27	2	1857.810	10781.939	0.75
28	1	1824.584	21455.761	0.75
N		28	28	28
Mean		1530.188	13715.974	0.744
SD		502.810	2669.003	0.195
Min		644.613	8632.172	0.500
Median		1429.822	13440.345	0.750
Max		2734.608	21455.761	1.330
Geometric Mean		1456.085	13473.043	0.721
CV% Geometric Mean		32.993	19.431	25.996

Table 15: Plasma Pharmacokinetic Parameters of digoxin for Treatment B

Subject	Period	C_{max} (ng/ml)	AUC_{0-96} (hr)*(ng/ml)	T_{max} (hr)
1	1	2313.039	12837.781	0.50
2	2	2309.434	14268.044	0.75
3	1	2571.660	17455.541	0.50
4	2	539.707	6373.657	3.50
5	1	2395.355	14545.854	0.75
6	2	2689.089	18584.836	0.75
7	1	1587.620	12877.001	1.33
8	2	2702.405	19406.321	0.75
9	1	3367.439	18282.739	1.00
10	2	2044.626	18327.511	1.00
11	1	2984.589	14949.542	0.50
12	2	2770.338	20020.183	1.00
13	2	2551.554	17133.898	1.00
14	1	2059.185	17168.285	0.75
15	1	2614.952	17022.316	0.50
16	2	2224.220	17944.812	1.00
17	2	816.105	11771.090	1.33
18	1	2361.896	13348.064	0.75
19	2	3075.660	17479.894	0.50
20	1	5044.130	23997.624	0.50
21	2	2807.843	17652.144	0.75
22	1	1618.290	11211.371	0.75
23	1	3399.330	22551.024	0.75
24	2	1536.919	12157.027	0.75
25	2	3545.195	22764.006	0.50
26	1	1726.667	16157.183	1.00
27	1	2014.773	13215.736	0.75
28	2	2449.415	23938.510	1.33
N		28	28	28
Mean		2432.908	16551.500	0.901
SD		870.562	4108.500	0.569
Min		539.707	6373.657	0.500
Median		2422.385	17151.091	0.750
Max		5044.130	23997.624	3.500
Geometric Mean		2254.118	15996.644	0.810
CV% Geometric Mean		45.789	28.453	44.310

Table 16: Urine Pharmacokinetic Parameters of digoxin for Treatment A

Subject	Period	Renal clearance (CLR)/Percent Recovered	unchanged drug excreted in urine (fe)/ Amount Recovered
1	2	34.631	86577.250
2	1	33.729	84321.800
3	2	147.642	369104.30
4	1	40.035	100086.65
5	2	44.327	110818.30
6	1	23.771	59428.600
7	2	69.431	173577.70
8	1	47.895	119737.65
9	2	51.154	127884.90
10	1	39.269	98171.600
11	2	129.333	323333.50
12	1	43.727	109317.10
13	1	50.636	126591.05
14	2	41.026	102565.70
15	2	79.524	198809.40
16	1	27.638	69094.500
17	1	42.759	106897.30
18	2	72.921	182302.70
19	1	33.563	83908.400
20	2	83.953	209883.35
21	1	31.465	78661.400
22	2	61.481	153703.00
23	2	57.658	144144.60
24	1	22.180	55449.800
25	1	35.214	88036.050
26	2	58.572	146429.75
27	2	78.807	197018.60
28	1	48.896	122241.10
N		28	28
Mean		54.687	136717.72
SD		29.158	72895.492
Min		22.180	55449.800
Median		46.111	115277.98
Max		147.642	369104.30
Geometric Mean		49.042	122606.02
CV% Geometric Mean		48.257	48.257

Table 17: Urine Pharmacokinetic Parameters of digoxin for Treatment B

Subject	Period	Renal clearance (CLR)/Percent Recovered	unchanged drug excreted in urine (fe)/ Amount Recovered
1	1	51.134	127834.30
2	2	82.111	205276.25
3	1	92.243	230606.45
4	2	33.465	83662.300
5	1	24.256	60641.150
6	2	55.153	137883.20
7	1	21.641	54103.200
8	2	85.335	213337.10
9	1	58.284	145709.60
10	2	81.090	202725.60
11	1	40.858	102145.85
12	2	60.708	151770.10
13	2	42.000	104998.80
14	1	36.688	91719.900
15	1	28.858	72144.000
16	2	50.049	125121.50
17	2	95.602	239003.90
18	1	40.477	101191.60
19	2	53.560	133899.30
20	1	51.236	128089.85
21	2	59.550	148874.30
22	1	39.662	99154.000
23	1	56.615	141538.50
24	2	30.860	77149.600
25	2	88.821	222053.60
26	1	35.062	87654.650
27	1	38.638	96595.200
28	2	60.642	151606.10
N		28	28
Mean		53.378	133446.07
SD		21.256	53141.044
Min		21.641	54103.200
Median		51.185	127962.08
Max		95.602	239003.90
Geometric Mean		49.410	123523.87
CV% Geometric Mean		42.201	42.201

14.3 SAFETY DATA

14.3.1 Displays of Adverse Events

There were no adverse events reported in this study.

14.3.2 Listing of Deaths, Other Serious and Significant Adverse Events

There were no deaths or serious adverse events in this study.

14.3.3 Narratives of Deaths, Other Serious and Certain Other Significant Adverse Events

There were no deaths, other serious and certain other significant adverse events recorded during the study.

14.3.4 Abnormal Laboratory Value Listing (each Subject)

Table 18: Abnormal Laboratory Value Listing

Parameter	Sub. No.	Pre-Study	Post Study	Remarks
Hematology	03	--	12.7 L	NS
	06	--	12.8 L	NS
	07	--	12.9 L	NS
	10	--	12.8 L	NS
	14	--	12.8 L	NS
	17	---	12.7 L	NS
	23	---	12.7 L	NS
	26	---	12.8 L	NS
RBC Count	05	---	4.4 L	NS
	09	---	4.4 L	NS

L= lower than normal Range, H: Higher than normal range, CS: Clinically Significant NS: Not Significant

15. REFERENCE LIST

1. Approved Protocol (Protocol No. C-CMP-SPI-001; Version 02, date: 07 Aug 2018)
2. Amendment No.:01 Date: 19 Dec 2018
3. ICH E3- Structure and content of Clinical Study Report

16. APPENDICES

16.1 STUDY INFORMATION

16.1.1 Protocol and Protocol Amendments

16.1.2 Sample case report form

16.1.3 List of IEC members along with approval letter - Representative written information for subject and sample informed consent documents

16.1.4 List and description of investigators and other important participants in the study, including brief CVs or equivalent summaries of training and experience relevant to the performance of the clinical study

16.1.5 Signatures of Principal or Coordinating Investigator(S) and the key personnel involved in the conduct of the study

16.1.6 Listing of subjects receiving test drug(s)/investigational product(s) from specific batches, where more than one batch was used

16.1.7 Randomization Schedule

16.1.8 Audit certificates

16.1.9 Documentation of statistical methods

16.1.10 Documentation of inter laboratory standardization methods and quality assurance procedures (If Used)

16.1.11 Publications based on the study

16.1.12 Important publications referenced in the report

16.2 SUBJECT DATA LISTINGS

16.2.1 Discontinued subjects

16.2.2 Protocol deviations

16.2.3 Subjects excluded from the efficacy analysis

16.2.4 Demographic data

16.2.5 Compliance and/or drug concentration data

16.2.6 Individual efficacy response data

16.2.7 Adverse event listings

16.2.8 Listing of individual laboratory measurements by subject when required by regulatory authority

16.3 CASE REPORT FORMS

16.3.1 CRFs for deaths, other serious adverse events and withdrawals for AE

16.3.2 Other CRFs submitted

16.4 INDIVIDUAL SUBJECT DATA LISTINGS

16.5 BIOANALYTICAL REPORT

16.0 APPENDICES

16.1 STUDY INFORMATION

16.1.1 PROTOCOL AND PROTOCOL AMENDMENTS

TITLE PAGE

Protocol Title

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

Protocol No.	:	C-CMP-SPI-001
Version	:	02
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Document status	:	Final
Content Final Date	:	07 Aug 2018

Sponsor	:	**CMP Development, LLC.** PO Box 147 8026 US Highway 264A Farmville, NC 27828 Telephone: (800)227-6637
CRO & **Clinical Study site**	:	**ClinSync Clinical Research Pvt. Ltd.** #4-1-1, Hayathnagar, Hyderabad, Telangana - 501505, India.

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Investigator's Declaration

I, the undersigned, have read and understood this protocol and hereby agree to conduct the study in accordance with this protocol and to comply with all the requirements regarding the obligations of investigators and all other pertinent requirements of the 'Good Laboratory Practice' ICH (Step 5) 'Guidance on Good Clinical Practice' and applicable regulatory requirements including applicable US FDA requirements for conduct of clinical studies.

I agree to comply with all relevant SOPs required for the conduct of this study. I further agree to ensure that all associates assisting in the conduct of this study are informed regarding their obligations.

Rsom — 07 Aug 2018

Signature — Date

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07 Aug 2018

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In charge - QA & RA,
ClinSync Clinical Research Pvt. Ltd.

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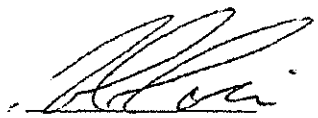
Sponsor's Approval

I, on behalf of CMP Development LLC, USA, have read, understood and approve this protocol. I agree to comply with all requirements regarding the obligations of sponsor and all other pertinent requirements of the 'Guidance on Good Laboratory Practice', ICH (Step 5) 'Guidance on Good Clinical Practice' and applicable regulatory requirements including applicable US FDA requirements for conduct of clinical studies.



8/9/18

Authorized Signatory

Date

Gerald Sakowski
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TABLE OF CONTENTS

VASAVI INSTITUTIONAL ETHICS COMMITTEE APPROVED

VASAVI INSTITUTIONAL ETHICS COMMITTEE APPROVED

VASAVI INSTITUTIONAL ETHICS COMMITTEE APPROVED

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SYNOPSIS

Title	An open label, balanced, randomized, single-dose, two-treatment, two-sequence, two-period, crossover oral drug-drug interaction study of spironolactone (perpetrator) and Digoxin (substrate drug) in healthy adult human subjects under fasting condition.
Objectives	*Primary objectives*: To characterize the pharmacokinetic profile of Digoxin (substrate drug) in the presence and absence of spironolactone (perpetrator) in healthy adult human subjects under fasting condition. *Secondary objective*: • To assess whether there is a drug-drug interaction with concomitant use of spironolactone. • To measure renal clearance (CL_R)/Percent Recovered and unchanged drug excreted in urine (f_e)/ Amount Recovered for digoxin in the presence and absence of spironolactone (perpetrator) in healthy adult human subjects under fasting condition. • To monitor safety of the subjects in drug-drug interaction study.
Study design	An open label, balanced, randomized, single-dose, two-treatment, two-sequence, two-period, crossover, drug-drug interaction study in healthy adult human subjects under fasting condition. Study Duration: In each period, subjects will be housed in a climate-controlled environment from at least 11.00 hours before starting of Day 1 until the collection of 96.00-hour blood sample on Day 10. No. of Subjects: 28 healthy adult human subjects will be included in the study. Additionally, 04 stand-by subjects will be included in order to dose 28 subjects in period I. Washout Period: At least 28 days.

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	No. of Urine & Blood samples: Urine & Blood samples collection will be started after the completion of dosing on Day 6. A total of 11 urine samples will be collected from each subject in each study period. Urine samples will be collected at intervals of (0.00-1.00), (1.00-2.00), (2.00-4.00), (4.00-6.00), (6.00-8.00), (8.00-12.00), (12.00-16.00), (16.00-24.00), (24.00-48.00), (48.00-72.00) and (72.00-96.00) hours post dose. Immediately out of the total volume of urine collected at each collection time interval, approximately 10 mL (2 x 5 mL aliquots) of urine samples will be transferred into two pre-labeled containers for analysis. They will be stored at deep freezer maintained at -20°C or lower until dispatched from Clinical Unit to Bioanalytical Department for analysis. A total of 25 blood samples will be collected from each subject in each study period. Blood samples will be collected at pre-dose (0.00) and post dose at 0.25, 0.50, 0.75, 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00, 36.00, 48.00, 60.00, 72.00 and 96.00 hours in each period. **Note:** Blood samples up to 3 hours post dose will be collected at bed side. Total Blood loss: The total blood loss per subject in the study will not exceed 292 mL for male/post-menopausal female subjects and 296 mL for female subjects of child bearing potential.
Investigational Products	Treatment (A): LANOXIN® (digoxin) USP 250 mcg (substrate drug). Treatment (B): LANOXIN® (digoxin) USP 250 mcg (substrate drug) + Spironolactone Oral Suspension 100 mg (Perpetrator; Carospir® Oral Suspension 20 mL of 25 mg / 5 mL of CMP Development LLC, USA.)

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Drug Administration	Spironolactone Oral Suspension 100 mg (Perpetrator; Carospir® Oral Suspension 20 mL of 25 mg / 5 mL of CMP Development LLC, USA.) will be administered as a single dose from Day 1 to Day 5 and Day 7 to Day 9, to the subjects who will be randomized to treatment B as per randomization scheme. On Day 6, after overnight fasting of at least 10.00 hours, either of treatment (A) or treatment (B) will be administered orally to the subjects in sitting posture with 240 ± 2 mL of water, at ambient temperature as per the randomization scheme. After a washout period of 28 days, the same procedure will be repeated during period-II. Subjects will be instructed not to chew or crush the tablet (for substrate drug) and swallow as a whole. Compliance for dosing will be assessed by a thorough check of the oral cavity immediately after dosing. A total of 240 ± 2 mL of water will be administered with investigational products. However, for Treatment B, 120 mL of water with 20 mL of CaroSpir oral suspension and 120 mL of water with one tablet of Lanoxin. *Instructions for CaroSpir oral suspension administration*: 1. CaroSpir oral suspension will be administered with a graduated syringe. 2. 20 mL of 25 mg / 5 mL of Carospir® Oral Suspension will be loaded in the syringes well in advance prior to dosing on the day of dosing. 3. The suspension in the syringe will be directly administered to the subjects. 4. The syringe will be rinsed with water (approximately 10 mL from 120 mL of dosing water) and will be administered to the subjects. This procedure will be followed till the syringe is free of the contents of the medication. Then the remaining

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	quantity of water (from the 120 mL) will also be administered to the subject. 5. Drug administration will be carried out at the same dosing times for all dosing days (i.e. from Day 01 to Day 09). 6. From Day 1 to 5 and Day 7 to 9 the Standard Breakfast will be served 0.50 hr (30 min) post dose.	
Clinical Safety measures	Safety will be determined by no clinically significant deviation from normal in findings of medical history, physical examination, electrocardiograms, vital signs, and clinical laboratory tests.	
Bioanalytical procedure	A validated LC-MS/MS method will be used for quantification of digoxin in presence of spironolactone and canrenone in plasma. Unchanged drug excreted in urine for digoxin will be analyzed by using validated LC-MS/MS method.	
PK parameters based on plasma data	Primary parameters	C_{max} and AUC_{0-96}
	Secondary parameters	T_{max}
PK parameters based on urine data	Renal clearance (CL_R) /Percent Recovered Unchanged drug excreted in urine (f_e)/Amount Recovered	
PK parameters evaluation using WinNonlin	Employing the estimated concentration vs. time profiles of digoxin using WinNonlin Software version 6.4 or above, the pharmacokinetic parameters will be calculated.	
Statistical analysis	Statistical analyses will be done using SAS® system for windows version 9.3 or above (SAS® Institute Inc., USA). The log-transformed pharmacokinetic parameters C_{max} and AUC_{0-96} will be analysed for Digoxin using ANOVA. The Intra subject CV, Power, Ratio analysis, and its 90% CI will be computed for log-transformed pharmacokinetic parameters C_{max} and AUC_{0-96} for digoxin.	
Criteria for	Absence of drug-drug interaction will be established if the 90%	

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evaluation	percent CI for the ratio of population geometric means between two treatments (A and B), based on log-transformed data, present in the equivalence limits of 80.00 - 125.00% for C_{max} and AUC_{0-96}. Summary statistics of pharmacokinetic profile of Digoxin (substrate drug) in the presence and absence of spironolactone (perpetrator) will be provided based on plasma and urine data. Renal clearance (CL_R)/Percent Recovered and unchanged drug excreted in urine (f_e)/ Amount Recovered for digoxin will also be measured and provided as a supporting data (evidence).

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
°C	Degree centigrade
ADR	Adverse Drug Reaction
AE	Adverse Event
ALT	Alanine Amino Transferase
ANOVA	Analysis of Variance
AST	Aspartate Amino Transferase
AUC_{0-96}	Area under the plasma concentration versus time curve from time 0 to the 96 hour time point concentration
BA/BE	Bio-Availability/Bio-Equivalence
BLQ	Below Limit of Quantification
BMI	Body Mass Index
BS	Biostatistics
CDSCO	Central Drugs Standard Control Organization
CL_R	Renal Clearance
C_{max}	Maximum measured plasma concentration
COA	Certificate of Analysis
CP	Clinical Pharmacology
CRF	Case Report Form
CSR	Clinical Study Report
CRO	Contract Research Organization
DDI	Drug-Drug Interaction
ESR	Erythrocyte sedimentation rate
fe	Unchanged drug excreted in urine
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
hr/hrs	Hours
ICD	Informed Consent Document
ICH	International Conference on Harmonization
ICMR	Indian Council of Medical Research
ICU	Intensive Care Unit
ID	Identity Document
IEC	Institutional Ethics Committee
IP	Investigational Product
IU	International Unit

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K_2EDTA	Di potassium Ethylene Diamine Tetraacetic Acid
Kg	Kilogram
LC-MS/MS	Liquid Chromatography tandem mass spectrometry
Ln	Natural Logarithm
LOQ	Limit of Quantification
Ltd.	Limited
M	Missing samples
Mcg	Microgram
Mg	Milligram
mL	Millilitre
MSE	Mean Square Error
MW	Medical Writing
No.	Number
NR	Not reportable
OTC	Over The Counter
P/A view	Posterior-Anterior view
PI	Principal Investigator
PK	Pharmacokinetic
RBC	Red Blood Corpuscles
Rh	Rhesus
Rpm	Rotations per minute
RPR	Rapid Plasma Reagin
SAE	Serious Adverse Event
SAS	Statistical Analysis System
SD	Standard Deviation
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SOP	Standard Operating Procedure
T_{max}	Time to achieve maximum plasma concentration
VDRL	Venereal Disease Research Laboratory
WBC	White Blood Corpuscles
βhCG	Beta Human Chorionic Gonadotropin

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1 STUDY INFORMATION

Protocol Title, Protocol Identifying Number, Date and Regulatory Submission		
Protocol Title	:	An open label, balanced, randomized, single-dose, two-treatment, two-sequence, two-period, crossover oral drug-drug interaction study of spironolactone (perpetrator) and Digoxin (substrate drug) in healthy adult human subjects under fasting condition.
Protocol Number	:	C-CMP-SPI-001, Version: 02
Date	:	07 Aug 2018
Regulatory Submission	:	USFDA
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Sponsor's Representative	:	Gerald Sakowski Chief Executive Officer CMP Development LLC, PO Box 147 8026 US Highway 264A, Farmville, NC 27828 Telephone: (800)227-6637
Monitors other than Sponsor	:	Not Applicable
Sponsor's Medical Expert		
Sponsor's Medical Expert	:	Dr. Kim A. Cook, M.D., M.S.P.H. Medical Director, Kiel Laboratories Inc, 5659 Southfield Drive, Flowery Branch, GA 30542 Telephone: 770-965-0006
Study Investigator		
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List of Facilities involved in the Study		
Clinical Facility and Screening	:	ClinSync Clinical Research Pvt. Ltd., Clinical Pharmacology Department,

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Facility		#4-1-1, Hayathnagar, Hyderabad, Telangana - 501505, India. Phone no.: +91-40-24203001/2/3 Fax no.: +91-40-24203000
Bioanalytical, Pharmacokinetic and Statistical Facility	:	ClinSync Clinical Research Pvt. Ltd., #4-1-1, Hayathnagar, Hyderabad, Telangana - 501505, India. Phone no.: +91-40-24203001/2/3 Fax no.: +91-40-24203000
Clinical Laboratory	:	Medcis Pathlabs India Pvt. Ltd. Plot 16 & 17, Swathi plaza, Bhavani Enclave, Anand Nagar, New Bowenpally, Secunderabad-500 011. Phone: 07702 29 29 29 Email: info@medcislabs.com
X-Ray Facility	:	SUNRISE HOSPITALS H. #: 4-9-321, Plot # 4 & 7, Hayathnagar, R.R. Dist, Hyderabad-501505. Phone no.: 9700009432 And/or ClinSync Clinical Research Pvt. Ltd., Clinical Pharmacology Department, #4-1-1, Hayathnagar, Hyderabad, Telangana - 501505, India.
Bio Medical Waste Management and Disposal	:	G.J. Multiclave (India) Pvt. Ltd., 7-1-47/1/A, D.K.Road, Ameerpet, Hyderabad-500 016, Telangana, India. Phone No.: 040 23756925, 30907999

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Emergency Services	:	SUNRISE HOSPITALS H. #: 4-9-321, Plot # 4 & 7, Hayathnagar, R.R. Dist, Hyderabad-501505. Phone no.: 9700009432
IEC	:	Vasavi Institutional ethics committee, Vasavi Medical Research Center #6-1-91, Lakdikapul, Khairatabad, Hyderabad, Telangana 500004 Phone no.: 040-65131333

2 BACKGROUND INFORMATION

Patients frequently use more than one medication at a time. Unanticipated, unrecognized, or mismanaged DDIs are an important cause of morbidity and mortality associated with prescription drug use and have occasionally caused the withdrawal of approved drugs from the market. In some instances, understanding how to safely manage a DDI may allow the FDA to approve a drug that would otherwise have an unacceptable level of risk.

The goal of a DDI study with pharmacokinetic endpoints is to inform clinical management strategies by determining whether there is a clinically significant increase or decrease in exposure to the substrate in the presence of the perpetrator. Clinical DDI studies compare substrate concentrations in the absence and presence of a perpetrator drug in vivo. The terms substrate is used to refer to the drug whose exposure may or may not be changed by a perpetrator drug. The term perpetrator refers to the drug that causes an effect on the substrate drug by inhibiting or inducing enzymes or transporters.

Types of DDI Studies

1. Prospective Studies and Retrospective Evaluations
2. DDI Studies with Index Perpetrators and Index Substrates
3. DDI Studies with Expected Concomitant Drugs

Index substrates and perpetrators are not chosen based on their use in the investigational drug's target population, but rather because of their well-defined interaction effects that provide information about the DDI potential of the

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investigational drug. Therefore, the results from DDI studies with index perpetrators or substrates are used to either extrapolate findings to concomitant medications sharing the same DDI properties or to help design DDI studies with commonly used concomitant medications in the investigational drug's target population. In contrast to DDI studies with index drugs, results from a concomitant-use study with a non-index drug can be difficult to extrapolate to other drugs.

The relevant concomitant medications for study include those used to treat the same condition for which the investigational drug is being studied or those used to treat common co-morbidities in the patient population.

The purpose of most DDI studies is to determine the ratio of a measure of substrate drug exposure (e.g., AUC ratio) in the presence and absence of a perpetrator drug.

Pharmacokinetics

Typical pharmacokinetic endpoints for DDI studies include changes in drug exposure parameters such as C_{max} and AUC_{0-72}. Pharmacokinetic sampling times should be sufficient to characterize the C_{max} and AUC_{0-72}. The sampling times for single-dose studies should be planned so that the mean difference between the C_{max} and AUC_{0-72} is less than 20 percent. Pharmacokinetic results of DDI study will be reported as the geometric mean ratio of the observed pharmacokinetic exposure measures with and without the perpetrator drug and include the associated 90 percent confidence interval. Observed variability of the interaction will also be reported.

Interpretation of DDI Study Results

The results of a DDI study are interpreted based on the no-effect boundaries for the substrate drug. No-effect boundaries represent the interval within which a change in a systemic exposure measure is considered not significant enough to warrant clinical action (e.g., dose or schedule adjustment, or additional therapeutic monitoring). When the 90 percent confidence intervals for systemic exposure ratios fall entirely within the equivalence range of 80 to 125 percent, the study concludes that there is no clinically significant DDI.

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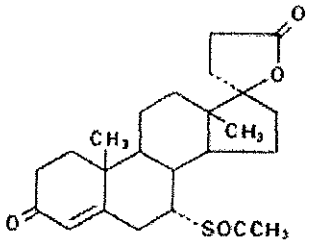
2.1 Spironolactone (CaroSpir®)

2.1.1 Description of Investigational Product

CaroSpir® (Spironolactone) Oral Suspension contains 25 mg of the aldosterone antagonist spironolactone, 17-hydroxy-7α-mercapto-3-oxo-17α-pregn-4-ene-21-carboxylic acid γ-lactone acetate per 5 mL, which has the following structural formula:



2.1.2 Pharmacology

Mechanism of Action

Spironolactone and its active metabolites are specific pharmacologic antagonists of aldosterone, acting primarily through competitive binding of receptors at the aldosterone-dependent sodium-potassium exchange site in the distal convoluted renal tubule. Spironolactone causes increased amounts of sodium and water to be excreted, while potassium is retained. Spironolactone acts both as a diuretic and as an antihypertensive drug by this mechanism. It may be given alone or with other diuretic agents that act more proximally in the renal tubule.

2.1.3 Pharmacokinetics

For an equivalent dose, Spironolactone Oral Suspension results in 15 to 37% higher serum concentration compared to Aldactone tablets. Information about the dose proportionality of spironolactone tablets is limited and, based on the results of studies comparing the suspension to tablets, doses of suspension higher than 100 mg might result in spironolactone concentrations that could be higher than expected.

Absorption

The peak plasma concentration (C_{max}) of spironolactone is reached 0.5 to 1.5 hours after dosing in healthy volunteers; for the active metabolite canrenone, the C_{max} is reached around 2.5 to 5 hours after dosing.

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Effect of food: A high fat and high calorie meal (57% of the ~1000 kcal of the meal was from fat) increased the bioavailability of spironolactone (as measured by AUC) by approximately 90%. Patients should establish a routine pattern for taking CaroSpir with regard to meals.

Distribution

Spironolactone and its metabolites are more than 90% bound to plasma proteins.

Metabolism

Spironolactone is rapidly and extensively metabolized. Metabolites can be divided into two main categories: those in which sulfur of the parent molecule is removed (e.g., canrenone) and those in which the sulfur is retained (e.g., TMS and HTMS). In humans, the potencies of TMS and 7-α-thiospirolactone in reversing the effects of the synthetic mineralocorticoid, fludrocortisone, on urinary electrolyte composition were approximately a third relative to spironolactone. However, since the serum concentrations of these steroids were not determined, their incomplete absorption and/or first-pass metabolism could not be ruled out as a reason for their reduced in vivo activities.

Elimination

The half-life of spironolactone is approximately 1- 2 hour, and the half-life of canrenone, 7-α-(thiomethyl) spirolactone (TMS), and 6-ß-hydroxy-7-α-(thiomethyl) spirolactone (HTMS) ranged from 10 to 35 hours.

Pharmacokinetic Parameters:

Parameters	Spironolactone	Canrenone
T_{max} (hours)	0.5 - 1.5	2.5 - 5
Mean terminal $T_{1/2}$ (hours)	1 - 2	10 - 35

2.1.4 Drug Interactions

Drugs and Supplements Increasing Serum Potassium

Concomitant administration of Spironolactone Oral Suspension with potassium supplementation or drugs that can increase potassium may lead to severe hyperkalemia. In general, discontinue potassium supplementation in heart failure patients who start Spironolactone Oral Suspension. Check serum potassium levels

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when ACE inhibitor or ARB therapy is altered in patients receiving Spironolactone Oral Suspension.

Examples of drugs that can increase potassium include:

- ACE inhibitors
- angiotensin receptor blockers
- aldosterone blockers
- non-steroidal anti-inflammatory drugs (NSAIDs)
- heparin and low molecular weight heparin
- trimethoprim

Lithium

Like other diuretics, Spironolactone Oral Suspension reduces the renal clearance of lithium, thus increasing the risk of lithium toxicity. Monitor lithium levels periodically when Spironolactone Oral Suspension is co-administered.

Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)

In some patients, the administration of an NSAID can reduce the diuretic, natriuretic, and antihypertensive effect of loop, potassium-sparing, and thiazide diuretics. Therefore, when Spironolactone Oral Suspension and NSAIDs are used concomitantly, monitor closely to determine if the desired effect of the diuretic is obtained.

Digoxin

Spironolactone and its metabolites interfere with radio immunoassays for digoxin and increase the apparent exposure to digoxin. It is unknown to what extent, if any, spironolactone may increase actual digoxin exposure. In patients taking concomitant digoxin, use an assay that does not interact with spironolactone.

Cholestyramine

Hyperkalemic metabolic acidosis has been reported in patients given spironolactone concurrently with cholestyramine.

Acetylsalicylic Acid

Acetylsalicylic acid may reduce the efficacy of spironolactone. Therefore, when Spironolactone Oral Suspension and acetylsalicylic acid are used concomitantly, Spironolactone Oral Suspension may need to be titrated to higher maintenance

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dose and the patient should be observed closely to determine if the desired effect is obtained.

2.1.5 Indications

Heart Failure: Spironolactone Oral Suspension is indicated for treatment of NYHA Class III-IV heart failure and reduced ejection fraction to increase survival, manage edema, and to reduce the need for hospitalization for heart failure.

Spironolactone Oral Suspension is usually administered in conjunction with other heart failure therapies.

Hypertension: Spironolactone Oral Suspension is indicated as an add-on therapy for the treatment of hypertension, to lower blood pressure in adult patients who are not adequately controlled on other agents. Lowering blood pressure reduces the risk of fatal and nonfatal cardiovascular events, primarily strokes and myocardial infarctions. These benefits have been seen in controlled trials of antihypertensive drugs from a wide variety of pharmacologic classes.

Edema caused by Cirrhosis: Spironolactone Oral Suspension is indicated for the management of edema in adult cirrhotic patients when edema is not responsive to fluid and sodium restriction.

2.1.6 Adverse Effects

Clinical Trials Experience

The following clinically significant adverse reactions are described in Warnings and precautions:

- Hyperkalemia
- Hypotension and Worsening Renal Function
- Electrolyte and Metabolic Abnormalities
- Gynecomastia
- Impaired neurological function/ coma in patients with hepatic impairment, cirrhosis and ascites

The following adverse reactions associated with the use of spironolactone were identified in clinical trials or post marketing reports. Because these reactions were reported voluntarily from a population of uncertain size, it is not always possible

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to estimate their frequency, reliably, or to establish a causal relationship to drug exposure.

Digestive: Gastric bleeding, ulceration, gastritis, diarrhea and cramping, nausea, vomiting.

Reproductive: Gynecomastia, decreased libido, inability to achieve or maintain erection, irregular menses or amenorrhea, postmenopausal bleeding, breast and nipple pain.

Hematologic: Leukopenia (including agranulocytosis), thrombocytopenia.

Hypersensitivity: Fever, urticaria, maculopapular or erythematous cutaneous eruptions, anaphylactic reactions, vasculitis.

Metabolism: Hyperkalemia, electrolyte disturbances, hyponatremia, hypovolemia.

Musculoskeletal: Leg cramps.

Nervous system /psychiatric: Lethargy, mental confusion, ataxia, dizziness, headache, drowsiness.

Liver / biliary: A very few cases of mixed cholestatic/hepatocellular toxicity, with one reported fatality, have been reported with spironolactone administration.

Renal: Renal dysfunction (including renal failure).

Skin: Stevens-Johnson Syndrome (SJS), toxic epidermal necrolysis (TEN), drug rash with eosinophilia and systemic symptoms (DRESS), alopecia, pruritis, chloasma.

2.1.7 Dosage & Administration, Contraindications and Warnings

Spironolactone Oral Suspension is not therapeutically equivalent to Aldactone. In patients requiring a dose greater than 100 mg, use another formulation. Doses of the suspension greater than 100 mg may result in spironolactone concentrations higher than expected.

Spironolactone Oral Suspension can be taken with or without food, but should be taken consistently with respect to food.

Contraindications

Spironolactone Oral Suspension is contraindicated for patients with the following conditions:

- Hyperkalemia

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- Addison's disease
- Concomitant use of eplerenone

Warning and precautions

Hyperkalemia

Spironolactone Oral Suspension can cause hyperkalemia. This risk is increased by impaired renal function or concomitant potassium supplementation, potassium-containing salt substitutes or drugs that increase potassium, such as angiotensin converting enzyme inhibitors and angiotensin receptor blockers.

Closer monitoring may be needed when Spironolactone Oral Suspension is given with other drugs that cause hyperkalemia or in patients with impaired renal function.

If hyperkalemia occurs, decrease the dose or discontinue Spironolactone Oral Suspension and treat hyperkalemia.

Hypotension and Worsening Renal Function

Excessive diuresis may cause symptomatic dehydration, hypotension and worsening renal function, particularly in salt-depleted patients or those taking angiotensin converting enzyme inhibitors and angiotensin II receptor blockers. Worsening of renal function can also occur with concomitant use of nephrotoxic drugs (e.g., aminoglycosides, cisplatin, and NSAIDs). Monitor volume status and renal function periodically.

Electrolyte and Metabolic Abnormalities

In addition to causing hyperkalemia, Spironolactone Oral Suspension can cause hyponatremia, hypomagnesemia, hypocalcemia, hypochloremic alkalosis, and hyperglycemia. Asymptomatic hyperuricemia can occur and rarely gout is precipitated. Monitor serum electrolytes, uric acid and blood glucose periodically.

Gynecomastia

Spironolactone Oral Suspension can cause gynecomastia. In RALES, patients with heart failure treated with a mean dose of 26 mg of spironolactone once daily, about 9% of the male subjects developed gynecomastia. The risk of gynecomastia increases in a dose-dependent manner with an onset that varies widely from 1-2 months to over a year. Gynecomastia is usually reversible.

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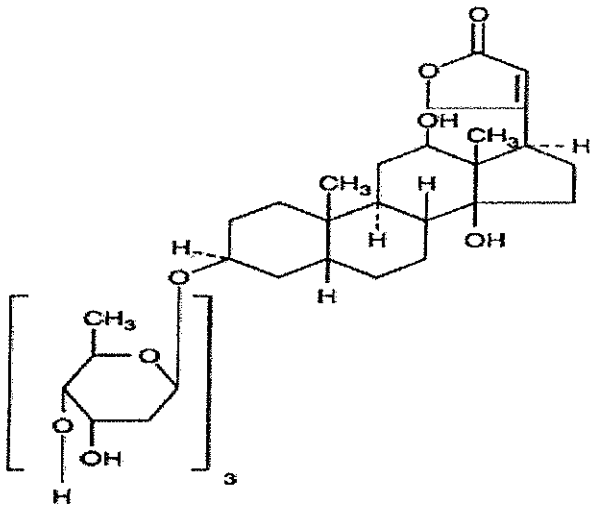
2.2 Digoxin (LANOXIN®)

2.2.1 Description of Investigational Product

Digoxin is the cardiac (or digitalis) glycoside, having effects on the myocardium. This drug is found in a number of plants. Digoxin is extracted from the leaves of *Digitalis lanata*. The term "digitalis" is used to designate the whole group of glycosides. The glycosides are composed of 2 portions: a sugar and a cardenolide (hence "glycosides").

Digoxin is described chemically as (3β,5β,12β)-3-[(O-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1→4)-O-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-ribo-hexopyranosyl)oxy]-12,14-dihydroxy-card-20(22)-enolide. Its molecular formula is $C_{41}H_{64}O_{14}$, its molecular weight is 780.95, and its structural formula is:



2.2.2 Pharmacology

Mechanism of Action

Digoxin inhibits sodium-potassium ATPase, an enzyme that regulates the quantity of sodium and potassium inside cells. Inhibition of the enzyme leads to an increase in the intracellular concentration of sodium and thus (by stimulation of sodium-calcium exchange) an increase in the intracellular concentration of calcium. The beneficial effects of digoxin result from direct actions on cardiac muscle, as well as indirect actions on the cardiovascular system mediated by effects on the autonomic nervous system. The autonomic effects include: (1) a

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vagomimetic action, which is responsible for the effects of digoxin on the sinoatrial and atrioventricular (AV) nodes; and (2) baroreceptor sensitization, which results in increased afferent inhibitory activity and reduced activity of the sympathetic nervous system and renin-angiotensin system for any given increment in mean arterial pressure.

2.2.3 Pharmacokinetics

Absorption

Following oral administration, peak serum concentrations of digoxin occur at 1 to 3 hours. Absorption of digoxin from Digoxin Tablets has been demonstrated to be 60% to 80% complete compared to an identical intravenous dose of digoxin (absolute bioavailability). When Digoxin Tablets are taken after meals, the rate of absorption is slowed, but the total amount of digoxin absorbed is usually unchanged. When taken with meals high in bran fiber, however, the amount absorbed from an oral dose may be reduced.

Distribution

Following drug administration, a 6- to 8-hour tissue distribution phase is observed. This is followed by a much more gradual decline in the serum concentration of the drug, which is dependent on the elimination of digoxin from the body. The peak height and slope of the early portion (absorption/distribution phases) of the serum concentration-time curve are dependent upon the route of administration and the absorption characteristics of the formulation.

Digoxin is concentrated in tissues and therefore has a large apparent volume of distribution. Digoxin crosses both the blood-brain barrier and the placenta.

Metabolism

Only a small percentage (16%) of a dose of digoxin is metabolized. The end metabolites, which include 3 ß-digoxigenin, 3-keto-digoxigenin, and their glucuronide and sulfate conjugates, are polar in nature and are postulated to be formed via hydrolysis, oxidation, and conjugation. The metabolism of digoxin is not dependent upon the cytochrome P-450 system, and digoxin is not known to induce or inhibit the cytochrome P-450 system.

Elimination

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Elimination of digoxin follows first-order kinetics (that is, the quantity of digoxin eliminated at any time is proportional to the total body content). Following intravenous administration to healthy volunteers, 50% to 70% of a digoxin dose is excreted unchanged in the urine. Renal excretion of digoxin is proportional to glomerular filtration rate and is largely independent of urine flow. In healthy volunteers with normal renal function, digoxin has a half-life of 1.5 to 2.0 days. The half-life in anuric patients is prolonged to 3.5 to 5 days.

Pharmacokinetic Parameters:

Parameters	Digoxin
T_{max} (hours)	1 - 3
Mean terminal $T_{1/2}$ (days)	1.5 – 2.0

2.2.4 Drug Interactions

Potassium-depleting diuretics are a major contributing factor to digitalis toxicity. Calcium, particularly if administered rapidly by the intravenous route, may produce serious arrhythmias in digitalized patients. Quinidine, verapamil, amiodarone, propafenone, indomethacin, itraconazole, alprazolam, and spironolactone raise the serum digoxin concentration due to a reduction in clearance and/or in volume of distribution of the drug, with the implication that digitalis intoxication may result. Erythromycin and clarithromycin (and possibly other macrolide antibiotics) and tetracycline may increase digoxin absorption in patients who inactivate digoxin by bacterial metabolism in the lower intestine, so that digitalis intoxication may result. Propantheline and diphenoxylate, by decreasing gut motility, may increase digoxin absorption. Antacids, kaolin-pectin, sulfasalazine, neomycin, cholestyramine, certain anticancer drugs, and metoclopramide may interfere with intestinal digoxin absorption, resulting in unexpectedly low serum concentrations. Rifampin may decrease serum digoxin concentration, especially in patients with renal dysfunction, by increasing the non-renal clearance of digoxin. There have been inconsistent reports regarding the effects of other drugs (e.g., quinine, penicillamine) on serum digoxin concentration. Thyroid administration to a digitalized, hypothyroid patient may increase the dose requirement of digoxin. Concomitant use of digoxin and sympathomimetics increases the risk of cardiac arrhythmias. Succinylcholine may

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cause a sudden extrusion of potassium from muscle cells, and may thereby cause arrhythmias in digitalized patients. Although calcium channel blockers and digoxin may be useful in combination to control atrial fibrillation, their additive effects on AV node conduction can result in advanced or complete heart block. Both digitalis glycosides and beta-blockers slow atrioventricular conduction and decrease heart rate. Concomitant use can increase the risk of bradycardia. Digoxin concentrations are increased by about 15% when digoxin and carvedilol are administered concomitantly. Therefore, increased monitoring of digoxin is recommended when initiating, adjusting, or discontinuing carvedilol.

Due to the considerable variability of these interactions, the dosage of digoxin should be individualized when patients receive these medications concurrently. Furthermore, caution should be exercised when combining digoxin with any drug that may cause a significant deterioration in renal function, since a decline in glomerular filtration or tubular secretion may impair the excretion of digoxin.

2.2.5 Indications

Heart Failure

Digoxin Tablet is indicated for the treatment of mild to moderate heart failure. Digoxin Tablet increases left ventricular ejection fraction and improves heart failure symptoms as evidenced by exercise capacity and heart failure-related hospitalizations and emergency care, while having no effect on mortality. Where possible, Digoxin Tablets should be used with a diuretic and an angiotensin-converting enzyme inhibitor, but an optimal order for starting these 3 drugs cannot be specified.

Atrial Fibrillation

Digoxin Tablets is indicated for the control of ventricular response rate in patients with chronic atrial fibrillation.

2.2.6 Adverse Effects

Clinical Trials Experience

In general, the adverse reactions of digoxin are dose-dependent and occur at doses higher than those needed to achieve a therapeutic effect. Hence, adverse reactions are less common when digoxin is used within the recommended dose range or

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therapeutic serum concentration range and when there is careful attention to concurrent medications and conditions.

Because some patients may be particularly susceptible to side effects with digoxin, the dosage of the drug should always be selected carefully and adjusted as the clinical condition of the patient warrants. In the past, when high doses of digoxin were used and little attention was paid to clinical status or concurrent medications, adverse reactions to digoxin were more frequent and severe.

Cardiac

Therapeutic doses of digoxin may cause heart block in patients with pre-existing sinoatrial or AV conduction disorders; heart block can be avoided by adjusting the dose of digoxin. Prophylactic use of a cardiac pacemaker may be considered if the risk of heart block is considered unacceptable. High doses of digoxin may produce a variety of rhythm disturbances, such as first-degree, second-degree (Wenckebach), or third-degree heart block (including asystole); atrial tachycardia with block; AV dissociation; accelerated junctional (nodal) rhythm; unifocal or multiform ventricular premature contractions (especially bigeminy or trigeminy); ventricular tachycardia; and ventricular fibrillation.

Gastrointestinal

Digoxin may cause anorexia, nausea, vomiting, and diarrhea. Rarely, the use of digoxin has been associated with abdominal pain, intestinal ischemia and hemorrhagic necrosis of the intestines.

CNS

Digoxin can produce visual disturbances (blurred or yellow vision), headache, weakness, dizziness, apathy, confusion, and mental disturbances (such as anxiety, depression, delirium, and hallucination).

Other

Gynecomastia has been occasionally observed following the prolonged use of digoxin. Thrombocytopenia and maculopapular rash and other skin reactions have been rarely observed.

Adverse experiences listed below for patients treated with Digoxin Tablets or placebo from 2 randomized, double-blind, placebo-controlled withdrawal trials.

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Patients in these trials were also receiving diuretics with or without angiotensin-converting enzyme inhibitors. These patients had been stable on digoxin, and were randomized to digoxin or placebo.

Cardiac: Palpitation, Ventricular extra systole, Tachycardia, Heart arrest.

Gastrointestinal: Anorexia, Nausea, Vomiting, Diarrhea, Abdominal pain

CNS: Headache, Dizziness, Mental disturbances.

Other: Rash, Death.

2.2.7 Dosage & Administration, Contraindications and Warnings

Dosage & Administration

Recommended dosages of digoxin may require considerable modification because of individual sensitivity of the patient to the drug, the presence of associated conditions, or the use of concurrent medications. In selecting a dose of digoxin, the following factors must be considered:

- The body weight of the patient. Doses should be calculated based upon lean (i.e., ideal) body weight.
- The patient's renal function, preferably evaluated on the basis of estimated creatinine clearance.
- The patient's age. Infants and children require different doses of digoxin than adults. Also, advanced age may be indicative of diminished renal function even in patients with normal serum creatinine concentration (i.e., below 1.5 mg/dL).
- Concomitant disease states, concurrent medications, or other factors likely to alter the pharmacokinetic or pharmacodynamic profile of digoxin.

Contraindications

Digitalis glycosides are contraindicated in patients with ventricular fibrillation or in patients with a known hypersensitivity to digoxin. A hypersensitivity reaction to other digitalis preparations usually constitutes a contraindication to digoxin.

Warning and precautions

Sinus Node Disease and AV Block

Because digoxin slows sinoatrial and AV conduction, the drug commonly prolongs the PR interval. The drug may cause severe sinus bradycardia or

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sinoatrial block in patients with pre-existing sinus node disease and may cause advanced or complete heart block in patients with pre-existing incomplete AV block. In such patients, consideration should be given to the insertion of a pacemaker before treatment with digoxin.

Accessory AV Pathway (Wolff-Parkinson-White Syndrome)

After intravenous digoxin therapy, some patients with paroxysmal atrial fibrillation or flutter and a coexisting accessory AV pathway have developed increased antegrade conduction across the accessory pathway bypassing the AV node, leading to a very rapid ventricular response or ventricular fibrillation. Unless conduction down the accessory pathway has been blocked (either pharmacologically or by surgery), digoxin should not be used in such patients. The treatment of paroxysmal supraventricular tachycardia in such patients is usually direct-current cardioversion.

Use in Patients with Preserved Left Ventricular Systolic Function

Patients with certain disorders involving heart failure associated with preserved left ventricular ejection fraction may be particularly susceptible to toxicity of the drug. Such disorders include restrictive cardiomyopathy, constrictive pericarditis, amyloid heart disease, and acute cor pulmonale. Patients with idiopathic hypertrophic subaortic stenosis may have worsening of the outflow obstruction due to the inotropic effects of digoxin. Digoxin should generally be avoided in these patients, although it has been used for ventricular rate control in the subgroup of patients with atrial fibrillation.

Use in Patients with Impaired Renal Function

Digoxin is primarily excreted by the kidneys; therefore, patients with impaired renal function require smaller than usual maintenance doses of digoxin. Because of the prolonged elimination half-life, a longer period of time is required to achieve an initial or new steady-state serum concentration in patients with renal impairment than in patients with normal renal function. If appropriate care is not taken to reduce the dose of digoxin, such patients are at high risk for toxicity, and toxic effects will last longer in such patients than in patients with normal renal function.

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Use in Patients with Electrolyte Disorders

In patients with hypokalemia or hypomagnesemia, toxicity may occur despite serum digoxin concentrations below 2.0 ng/mL, because potassium or magnesium depletion sensitizes the myocardium to digoxin. Therefore, it is desirable to maintain normal serum potassium and magnesium concentrations in patients being treated with digoxin. Deficiencies of these electrolytes may result from malnutrition, diarrhea, or prolonged vomiting, as well as the use of the following drugs or procedures: diuretics, amphotericin B, corticosteroids, antacids, dialysis, and mechanical suction of gastrointestinal secretions.

Hypercalcemia from any cause predisposes the patient to digitalis toxicity. Calcium, particularly when administered rapidly by the intravenous route, may produce serious arrhythmias in digitalized patients. On the other hand, hypocalcemia can nullify the effects of digoxin in humans; thus, digoxin may be ineffective until serum calcium is restored to normal. These interactions are related to the fact that digoxin affects contractility and excitability of the heart in a manner similar to that of calcium.

Use in Thyroid Disorders and Hypermetabolic States

Hypothyroidism may reduce the requirements for digoxin. Heart failure and/or atrial arrhythmias resulting from hypermetabolic or hyperdynamic states (e.g., hyperthyroidism, hypoxia, or arteriovenous shunt) are best treated by addressing the underlying condition. Atrial arrhythmias associated with hypermetabolic states are particularly resistant to digoxin treatment. Care must be taken to avoid toxicity if digoxin is used.

Use in Patients with Acute Myocardial Infarction

Digoxin should be used with caution in patients with acute myocardial infarction. The use of inotropic drugs in some patients in this setting may result in undesirable increases in myocardial oxygen demand and ischemia.

Use During Electrical Cardioversion

It may be desirable to reduce the dose of digoxin for 1 to 2 days prior to electrical cardioversion of atrial fibrillation to avoid the induction of ventricular arrhythmias, but physicians must consider the consequences of increasing the

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ventricular response if digoxin is withdrawn. If digitalis toxicity is suspected, elective cardioversion should be delayed. If it is not prudent to delay cardioversion, the lowest possible energy level should be selected to avoid provoking ventricular arrhythmias.

Use in Patients with Myocarditis

Digoxin can rarely precipitate vasoconstriction and therefore should be avoided in patients with myocarditis.

Use in Patients with Beri Beri Heart Disease

Patients with beri beri heart disease may fail to respond adequately to digoxin if the underlying thiamine deficiency is not treated concomitantly.

3 STUDY OBJECTIVES AND PURPOSE

3.1 Objectives

Primary Objective: To characterize the pharmacokinetic profiles of Digoxin (substrate drug) in the presence and absence of spironolactone (perpetrator) in healthy adult human subjects under fasting condition.

Secondary objective:

To assess whether there is a drug-drug interaction with concomitant use of spironolactone.

To measure renal clearance (CL_R)/Percent Recovered and unchanged drug excreted in urine (f_e)/ Amount Recovered for digoxin in the presence and absence of spironolactone (perpetrator) in healthy adult human subjects under fasting condition.

To monitor safety of the subjects in drug-drug interaction study.

3.2 Purpose

CaroSpir® is an oral suspension of spironolactone approved by FDA. During the approval process, FDA recommended the sponsor, CMP Development LLC, USA, to conduct a drug-drug interaction of spironolactone oral suspension with Digoxin. Hence, this Drug-Drug Interaction Study of spironolactone oral suspension with Digoxin is planned with the stated objective to assess whether there is a drug-drug interaction resulting in increased digoxin levels with concomitant use of spironolactone.

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4 STUDY DESIGN

An open label, balanced, randomized, single-dose, two-treatment, two-sequence, two-period, crossover, drug-drug interaction study in healthy adult human subjects under fasting condition.

Schematic Diagram of Study Design, Procedures and Stages are attached as Appendix I.

4.1 Number of Subjects

28 healthy adult human subjects will be included in the study. Additionally, 04 stand-by subjects will be included in order to dose 28 subjects in period I.

4.2 Randomization

The order of receiving the treatment A and treatment B for each subject during the study will be determined randomly. According to the randomization schedule, subjects will receive the assigned formulation in each period, with the possible sequences 'AB' or 'BA' (where A = substrate and B = substrate & perpetrator). Equal allocation of subjects to each sequence will be ensured. The randomization will be balanced and the randomization schedule as well as the dispensing records will be kept in the drug store under controlled access. The study personnel involved in dispensing (pharmacist) and the Principal Investigator will be accountable for ensuring compliance to randomization schedule. Randomization schedule will be generated using SAS® software 9.3 or higher.

4.3 Blinding

The study will be conducted as open label study. The study personnel involved in the sample analysis will be kept blinded from the randomization schedule during entire study.

4.4 Housing and Washout period

The subjects will be housed for at least 11.00 hours prior to drug administration on Day 1 untill the collection of 96.00 hours blood sample on Day 10. There will be a washout period of at least 28 days between each consecutive treatment periods.

5 INVESTIGATIONAL PRODUCTS

Treatment (A): LANOXIN® (digoxin) USP 250 mcg (substrate drug)

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Treatment (B): LANOXIN® (digoxin) USP 250 mcg (substrate drug) + Spironolactone Oral Suspension 100 mg (perpetrator; Carospir® Oral Suspension 20 mL of 25 mg / 5 mL of CMP Development LLC, USA.)

5.1 Receipt, Storage and Accountability of Investigational Products

5.1.1 Receipt of Investigational Products

Sponsor should supply sufficient quantity of investigational products, for administration to subjects as well as retention purpose. The perpetrator drug product (Spironolactone) will be supplied in PET bottles with child resistant caps and induction seal whereas substrate drug product (Digoxin) will be supplied in sealed packages and/or strips with appropriate label and certificates of analysis.

5.1.2 Storage of Investigational Products

After receipt of the investigational products, they will be transferred to the pharmacy. The investigational products will be stored as per the storage conditions of investigational products in the pharmacy.

5.1.3 Accountability of Investigational Products

Accountability for the investigational products will be documented in the respective "Dispensing and accountability of IP's" record for the substrate and perpetrator drug products as per applicable ClinSync SOP.

5.1.4 Dispensing

The Pharmacist is responsible for dispensing and will dispense a quantity of the substrate and perpetrator drug products sufficient for dosing along with stand-by units for the period as per the randomization schedule and the remaining will be kept in their original containers as retention samples. As Carospir® is suspension, Pharmacist will shake the bottle well (by means of inverting the bottle and shaking vigorously for at least 15 seconds) just before dispensing and the same will be documented. The dispensed doses will be transferred to the drug-dispensing sachets (for tablet) and unit dose container (for suspension) and pre-labeled (sample format) as shown below:

Project No:	Period No:
Drug name:	Treatment:
Subject No:	Exp. Date:

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Batch/Lot No:	Dosage Form:
Qty. x strength:	
Storage conditions:	
Dispensed by:_____	
Administered by:_____	
(For BA/BE Study Use Only)	

5.1.5 Handling of Unused Investigational Products

The dispensed but un-dosed investigational product (tablets/substrate drug product) will be kept along with the remaining investigational product after completion of the project. Dispensed but un-dosed investigational product (suspension/perpetrator drug product) will be discarded as per in-house SOP.

5.1.6 Retention of Samples

After completion of the study, remaining investigational products will be retained in the pharmacy as per applicable ClinSync SOP.

6 TREATMENT OF SUBJECTS

6.1 Treatments to be administered

Treatment A		
Name of the Product	:	LANOXIN® (digoxin) USP 250 mcg
Dose	:	250 mcg
Dosing Schedule	:	One tablet as a single dose to each subject
Route/Mode of Administration	:	Orally with 240 ± 2 mL of water
Treatment Period	:	Single dose for 1 Day (day 6) as per randomization schedule
Treatment B		
Name of the Product	:	LANOXIN® (digoxin) USP 250 mcg + Spironolactone Oral Suspension 100 mg (CaroSpir® Oral Suspension 20 mL of 25 mg / 5 mL)
Dose	:	250 mcg of digoxin and 100 mg of spironolactone as 20 mL of 25 mg / 5 mL

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Dosing Schedule	:	One tablet of digoxin and 20 mL of 25mg/5mL CaroSpir oral suspension as a single dose to each subject
Route/Mode of Administration	:	Orally with about 240 ± 2 mL of water
Treatment Period	:	Single dose for 9 Days as per randomization schedule

6.2 Drug Administration

Spironolactone Oral Suspension 100 mg (Perpetrator; Carospir® Oral Suspension 20 mL of 25 mg / 5 mL of CMP Development LLC, USA.) will be administered as a single dose from Day 1 to Day 5 and Day 7 to Day 9, to the subjects who will be randomized to treatment B as per randomization scheme.

On Day 6, after overnight fasting of at least 10.00 hours, either of treatment (A) or treatment (B) will be administered orally to the subjects in sitting posture with 240 ± 2 mL of water, at ambient temperature as per the randomization scheme. After a wash out period of 28 days, the same procedure will be repeated during period-II.

Subjects will be instructed not to chew or crush the tablet (for substrate drug) and swallow as a whole.

Compliance for dosing will be assessed by a thorough check of the oral cavity immediately after dosing.

A total of 240 ± 2 mL of water will be administered with investigational products. However, for Treatment B, 120 mL of water with 20 mL of CaroSpir oral suspension and 120 mL of water with one tablet of Lanoxin.

Instructions for suspension administration:

1. CaroSpir oral suspension will be administered with a graduated syringe.
2. 20 mL of 25 mg / 5 mL of Carospir® Oral Suspension will be loaded in the syringes well in advance prior to dosing on the day of dosing.
3. The suspension in the syringe will be directly administered to the subjects.
4. The syringe will be rinsed with water (approximately 10 mL from 120 mL of dosing water) and will be administered to the subjects. This procedure will be followed till the syringe is free of the contents of the medication. Then the remaining quantity of water (from the 120 mL) will also be administered to the subject.

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5. Drug administration will be carried out at the same dosing times for all dosing days (i.e. from Day 01 to Day 09).
6. From Day 1 to 5 and Day 7 to 9, the Standard Breakfast will be served at 0.50 hr (30 min) post dose.

6.3 Sampling Schedule

Urine & Blood samples collection will be started after the completion of dosing on Day 6.

A total of 11 urine samples will be collected from each subject in each study period.

Urine samples will be collected at intervals of (0.00-1.00), (1.00-2.00), (2.00-4.00), (4.00-6.00), (6.00-8.00), (8.00-12.00), (12.00-16.00), (16.00-24.00), (24.00-48.00), (48.00-72.00) and (72.00-96.00) hours post dose.

Immediately out of the total volume of urine collected at each collection time interval, approximately 10 mL (2 x 5 mL aliquots) of urine samples will be transferred into two pre-labeled containers for analysis. They will be stored at deep freezer maintained at -20°C or lower until dispatched from Clinical Unit to Bioanalytical Department for analysis.

A total of 25(1 × 5 mL) blood samples will be collected from each subject in each study period. Blood samples will be collected at pre-dose (0.00) and post dose at 0.25, 0.50, 0.75, 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00, 36.00, 48.00, 60.00, 72.00 and 96.00 hours post dose in each period.

Note: Blood samples up to 3 hours post dose will be collected at bed side.

6.4 Sampling Procedure

Immediately out of the total volume of urine collected at each collection time interval, approximately 10 mL (2 x 5 mL aliquots) of urine samples will be transferred into two pre-labeled containers and will be stored at deep freezer maintained at -20°C or lower until dispatched from Clinical Unit to Bioanalytical Department for analysis. The urine samples will be stored at -20°C or lower in the Bioanalytical facility until analysis. After collecting the urine samples from all the

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subjects at each collection time interval, the total pooled volume will be noted down.

The urine samples will be transferred to analytical site for analysis after the completion of clinical phase.

The pre and post-dose blood samples up to 24.00 hrs will be collected via an indwelling cannula place in an antecubital vein or one of the forearm veins. Heparin-lock technique (about 0.5 mL of heparinized saline [1 ml of 5 IU/ml heparin in normal saline solution] will be injected into the cannula after each sample collection) will be used to prevent clotting of the blood in the indwelling cannula. Before each blood sample is drawn, 0.5 mL of blood will be discarded (except 36.00, 48.00, 60.00, 72.00 and 96.00 post dose samples and if the blood is being collected by a fresh vein-puncture) to prevent the heparin in the cannula from interfering with the analysis.

At each time point, the blood samples will be collected in pre-labeled (Project No., Subject No, Period No, sampling time point and Sample ID) vacutainers® containing K_2EDTA as anticoagulant. If for any reason the indwelling cannula is blocked or must be removed for practical reasons, the blood sample will be drawn by inserting a fresh cannula after removal of the blocked cannula or by direct venipuncture, as decided by the investigator. If there are more than 2 draws that need to be done after the cannula is blocked, a fresh cannula preferably will be inserted for that subject.

The pre-dose blood sample will be collected within 2 hours before dosing and the post-dose in-house samples will be collected with a window period of + 2 minutes from the scheduled sampling time.

During collection of blood sample at each time point the mid-point of the minute will be considered to calculate the nearest minute, which will be recorded on the appropriate form. The deviations greater than mentioned in this protocol from the scheduled sampling time will be reported as protocol deviations.

The actual time points will be taken into account for the Pharmacokinetic Analysis if any deviations occur for blood collection.

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After collecting the blood samples from all the subjects at each sampling time point, samples will be centrifuged for all pharmacokinetic samples. The collected blood samples will be kept on ice-packs until centrifugation. The samples will be centrifuged within 45 minutes from the scheduled blood sample collection. The samples will be centrifuged at the set conditions of 4000 rpm for 10 minutes at 4°C to separate plasma. The separated plasma will be transferred to pre-labeled polypropylene tubes in two equal aliquots.

The plasma samples will be then stored upright in a box containing dry ice or in a freezer at a temperature of -20 ± 5°C for interim storage (pre-dose and post-dose samples up to 4.00 hours) and then will be transferred to -70 ± 15°C at the clinical site.

The plasma samples will be transferred from cold chain management (CCM) department to bioanalytical facility for analysis batch wise as per approved sequence schedule, after the completion of clinical phase.

In case meal, urine collection, blood sample collection timings, ECG and vital signs coincide, blood samples first and then Urine sample, vitals, ECG followed by meals.

6.5 Blood Loss

The total blood loss per subject in the study will not exceed 292 mL for male/post-menopausal female subjects and 296 mL for female subjects of child bearing potential. In addition to the above, about 3-5 mL of blood will be collected for repeat post study safety assessment whenever needed or as decided by the Physician/Clinical Investigator/Principal Investigator.

	PK samples for two periods (50 samples of 5 mL each)	:	250 mL
+	Discarded heparin containing blood for two periods (40 x 0.5 mL)	:	20 mL
+	Blood withdrawn for screening	:	09 mL
+	Blood sample collection for Creatinine clearance and serum potassium for two periods (02 x 03 mL)	:	06 mL
+	Blood sample collection for post-study safety assessment	:	07 mL
	Total Blood Loss for male/post-menopausal female subjects	:	292 mL

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+	Serum pregnancy test for two periods (2 x 02 mL)	:	04 mL
	Total Blood Loss for female subjects of child bearing potential	:	296 mL

6.6 Diet and Water

Subjects will be fasted for at least 10.00 hours prior to drug administration on each dosing day. Standard diet will be provided in each period to all subjects.

Day 1 to Day 9 (except on Day 6):

On each day from day 1 to day 9 (except on Day 6), the standard breakfast, Lunch, Snacks and dinner will be served at 0.50, 4.00, 8.00, and 12.00 hours post dose.

Day 6: On day 6, lunch, snacks and dinner will be served at 4.00, 8.00, and 12.00 hours post dose.

Note:

- On Day 6, subjects will be fasted for at least 10.00 hours prior to dosing.

Days	Breakfast	Lunch	Snacks	Dinner
	Time post dose (hours)			
Day 1 to Day 5	0.50 (30 min)	04.00	08.00	12.00
Day 6	NA	04.00	08.00	12.00
Day 7 to Day 9	0.50 (30 min)	04.00	08.00	12.00
Dinner on day '5' will be completed at least 10.00 hours prior to dosing				

Water will be provided *ad libitum* during the study, except one hour before and one hour after dosing on Day 6.

Note: On all days' subjects will be instructed to consume more water (i.e. at least 4-5 liters per day) to control the hypotension and volume depletion.

6.7 Total duration of subject participation in the study

- Screening: Screening will take place within 28 days before the start of the study.
- Dosing: Day 1 to 9 in each period (Including a gap of at least 28 days between two successive treatment schedules of the study as wash out period)
- Follow up: Adverse event will be considered as related to the study if noticed or reported before study completion date and will be followed up till resolution.

VASAVI INSTITUTIONAL ETHICS COMMITTEE APPROVED

- The study duration will be for approximately 39 days, which shall include from period I check-in to post study safety assessment of the study.

6.8 Maintenance of Study Treatment Randomization Schedule

The randomization schedule will be made available to the treating physician, the Principal Investigator, the Sponsor, the IEC and/or the Regulatory Authority in case of any serious adverse event to ascertain the treatment allocation.

6.9 Documentation of Study Activities

All the study related activities will be entered directly in the individual activity forms. All the raw data and source documents will be compiled as such.

7 SELECTION AND WITHDRAWAL OF SUBJECTS

All subjects will undergo a screening procedure comprising of clinical examination, recording of electrocardiogram, 2D Echocardiogram, and laboratory investigations of blood as well as urine, which will be valid for the number of 28 days from the day of screening. Radiological investigations (Chest X-Ray) will be repeated, if not done in the past 6 months or, if clinically indicated at the time of screening. The subjects will be selected on the basis of the following inclusion and exclusion criteria.

7.1 Subject Inclusion Criteria

- Healthy human subjects aged between 20 and 35 years (including both).
- Subjects with a BMI between 18.5 - 24.9 Kg/m^2 (including both) but body weight not less than 60 Kgs.
- Subjects who were screened at least 48 hours prior to check-in
- Subjects with normal health as determined by personal medical history, clinical examination, and laboratory examinations including serological tests during the screening as per section 11.2
- Subjects with normal 2D echo
- Subjects having normal 12-lead electrocardiogram (ECG) or ECG with no clinical significant abnormalities as determined by Investigator.
- Subjects having normal chest X-Ray (P/A view) or chest X-ray with no clinically significant abnormalities as determined by investigator.
- Subjects able to communicate effectively.

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- Subjects willing to give written informed consent and adhere to all the requirements of this protocol.
- **Additional inclusion criteria for female subjects,**
  - Female of childbearing potential practicing an acceptable method of birth control for the duration of the study as judged by the investigator(s), such as condoms, foams, jellies, diaphragm, intrauterine device (IUD), or abstinence: or
  - Postmenopausal for at least 1 year, or
  - Surgically sterile (bilateral tubal ligation, bilateral oophorectomy or hysterectomy has been performed on the subject).

7.2 Subject Exclusion Criteria

- Subjects having contraindications or hypersensitivity to study drug or related group of drugs.
- History or presence of any medical condition or disease according to the opinion of the physician.
- History or presence of significant cardiovascular, pulmonary, hepatic, renal, gastrointestinal, endocrine, immunological, dermatological, neurological or psychiatric disease or disorder.
- Subject having QT/QTc interval >450 milliseconds
- History or presence of significant alcoholism or drug abuse in the past one year.
- History or presence of significant smoking (more than 10 cigarettes or beedies /day or consumption of tobacco products).
- Subjects who fail to abstain from consuming any alcoholic products from 48.00 hours prior to check-in to till check-out / last sample of the study.
- Subjects who fail to abstain from any xanthine-containing food and/or beverages (like chocolate, tea, coffee, cola drinks), cigarettes and tobacco containing products and grapefruit and/or it's juice from 48.00 hours prior to check-in to till check-out / last sample of the study.

VASAVI INSTITUTIONAL ETHICS COMMITTEE APPROVED

- Subjects who fail to refrain from pan or pan masala, gutkha, masala (containing beetle nut and tobacco) for 48.00 hours prior to check-in to till check-out/last sample of the study.
- Difficulty with donating blood.
- Systolic blood pressure less than 110 mm Hg or more than 140 mm Hg.
- Diastolic blood pressure less than 70 mm Hg or more than 90 mm Hg.
- Pulse rate less than 60 beats/minute or more than 100 beats/minute.
- Use of any prescribed medication during last two weeks or OTC medicinal products/ herbal products during the last one week prior to check-in.
- Major illness during 90 days before check-in.
- Participation in a drug research study within past 90 days of check-in.
- Donation of blood (i.e. one unit or 350 mL) in the past 90 days before check-in.
- History of unusual diet consumption in the past 3 weeks before check-in.
- **Additional exclusion criteria for female subjects,**
  - Volunteer demonstrating a positive pregnancy test.
  - Volunteers who are pregnant, currently breast-feeding or who are likely to become pregnant during the study.
  - Volunteers who have used implanted or injected hormonal contraceptives anytime during the 6 months prior to study or used hormonal contraceptives within 14 days before dosing.

7.3 Criteria for Discontinuation of the study

The Sponsor reserves the right to discontinue the study at any time. The Principal Investigator reserves the right to discontinue the study for safety reasons at any time. The IEC may ask to terminate the study, if there are major violations of the ethical considerations or due to any serious adverse event(s). Reasons for the termination of the study will be provided to the subjects.

7.4 Tests to be performed before Check-in of the study

Subject's urine (approximately 20 mL) will be screened for drugs of abuse like cocaine, cannabis, amphetamines, barbiturates, benzodiazepines and opiates at the

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time of each check-in of study. Subject will be rejected/ withdrawn from the study at any point of the time if the result is positive for these drugs.

Alcohol breath test will be performed for all subjects before check-in into each period of the study. Subject will be rejected/withdrawn from the study if the result is positive for alcohol.

Serum Pregnancy test (β-hCG) will be performed for all the female subjects having child bearing potential and urine pregnancy test will be performed for post-menopausal subjects at every check in. Subjects will be rejected/withdrawn from the study if result is positive for pregnancy at any point of time.

7.5 Subject Withdrawal Criteria

7.5.1 Procedures to Withdraw Subjects from the Investigational Product Treatment

- Subject wishes to withdraw consent on his own accord in person and the same will be considered as dropout.

The Principal Investigator/Clinical Investigator may withdraw a subject from the study for any of the following:

- The subject is non-cooperative and non-compliant.
- The subject is found to have entered the study in violation of this protocol.
- If any subject experience emesis at or before 2 times of median T_{max} on Day 6.
- The subject is suffering from any other clinically significant adverse event.
- The subject who receives/requires any concomitant medication, which may interfere with the pharmacokinetic property of the study drug.
- The subject who underwent/requires hospitalization during the course of the study.
- The subject reported to the clinical facility for check-in after the prescribed time limit there by failing to meet the protocol requirements.
- If it is felt in the investigator's opinion that it is not in the subject's best interest to continue.
- The subject is tested positive for Alcohol breath test.
- The subject is tested positive for any one of the urine drugs of abuse.
- Subjects who miss 03 consecutive blood draws in a study period.

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7.5.2 The Type and Timing of the Data to be collected for Withdrawn Subjects

Any subject withdrawal during the study along with the reason thereof shall be documented in the Form for Subject Withdrawal/ Drop out Record, as per applicable ClinSync SOP.

The samples of subjects withdrawn due to adverse events will also be analyzed. Plasma concentration vs. time data of that subject will be tabulated separately and reported in the study report and will not be included in pharmacokinetic and statistical analysis.

The concentrations of Unchanged drug excreted in urine/Amount Recovered for digoxin will be tabulated separately and reported in the study report for all withdrawn subjects due to adverse events.

7.5.3 Replacement of Subjects

No dosed subject will be replaced. 04 additional standby subjects will be enrolled to ensure dosing 28 subjects in period I. Any subject withdrawn/dropped out from the study due to any reason prior to dosing of period I will be replaced by stand-by subject. If there are no dropouts, these 04 standby subjects will be checked out after completion of 28 subjects dosing in period I. All details will be documented appropriately.

Note: In period I, two standby subjects will be checked out from the study on day 1 after completion of the dosing activity and remaining two standby subjects will be checked out from the study on day 6 after completion of the dosing activity.

7.5.4 The Follow-up for Subjects Withdrawn from Investigational Product Treatment

Any subject withdrawn during the study due to any adverse event will be followed up wherever possible till resolution or until the physician believes that there will be no further change. This may involve additional visits.

7.6 Restrictions

7.6.1 Diet

Subject should not take any unusual diet (for e.g. low salt diet) at least 3 weeks prior to check-in and throughout the study.

VASAVI INSTITUTIONAL ETHICS COMMITTEE APPROVED

Subjects will be instructed to abstain from consuming any alcoholic products from 48.00 hours prior to check-in to till check-out / last sample of the study. They will not be allowed to have any xanthine-containing food and/or beverages (like chocolate, tea, coffee, cola drinks), cigarettes and tobacco containing products and grapefruit and/or it's juice from 48.00 hours prior to check-in to till check-out / last sample of the study.

7.6.2 Posture

Subjects who are dosed will remain in a supine position or semi recumbent position for 03 hours post-dose on Day 06 and only necessary movement will be allowed during this period. Thereafter subject will be allowed to ambulate freely during the remaining part of the study. Subject will not be allowed to sit down (except as directed by the physician secondary to adverse events) during restriction period.

Note:

- In case any subject has any adverse event and requires any change in restrictions of water & posture, it will be done after consultation with the Principal Investigator/Attending Physician.
- During the posture restriction period all study related activities (Blood and, vital signs measurement) will be done at bed side only, except urine sample collection.

7.6.3 Medication

Subjects will be instructed not to take any prescription medications within two weeks prior to check-in and throughout the study. Subjects will be instructed not to take any OTC products, herbal medications, etc. within one week prior to check-in and throughout the study. If these medications are required, subject will not be enrolled or may be withdrawn from study by investigator.

7.6.4 Others

Subjects will be instructed to refrain from pan or pan masala, gutkha, masala (containing beetle nut and tobacco) for 48.00 hours prior to check-in to till check-out/last sample of the study.

VASAVI INSTITUTIONAL ETHICS COMMITTEE APPROVED

8 BIOANALYTICAL PROCEDURES

8.1 Method Description

a. The bioanalytical method will be developed & validated by using LC-MS/MS for quantification of digoxin in presence of spironolactone and canrenone as per current version of in-house SOP "Bioanalytical Method Validation" and applicable regulatory requirements.

b. Samples will be assayed for digoxin in presence of spironolactone and canrenone using the validated method at ClinSync Clinical Research Pvt. Ltd., Hyderabad.

c. Unchanged drug excreted in urine for digoxin will be analyzed by using validated LC-MS/MS method.

d. Analysis of the plasma will include distribution of quality control samples throughout each batch of study samples. All the available plasma samples will be analyzed in bio-analytical laboratory.

e. However, the samples of all withdrawn and dropped out subjects will be analyzed and tabulated in a separate table.

f. The analyst will not have access to the randomization code list during the course of the analysis.

g. All concentration values below the limit of quantification (BLQ) will be set to zero for obtaining descriptive statistics. All zero pre-dose concentrations will not be reported as BLQ

h. Whenever possible, all samples from each subject will be analyzed on the same standard curve. Samples with drug concentration greater than the upper limit of the validated range of the assay will be diluted with the appropriate drug-free biological fluid and method validation and repeat analysis will be done as per the current version of applicable ClinSync SOP.

i. Incurred sample reanalysis (ISR) will be performed as per current version of applicable ClinSync SOP.

9 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

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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 non-compartmental model of Phoenix WinNonlin Professional Software Version 6.4 (Pharsight Corporation, USA) or above.

Pharmacokinetic Parameters for digoxin in plasma:

Primary Pharmacokinetic parameters

C_{max} : Maximum measured plasma concentration.

AUC_{0-96} : 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

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

Pharmacokinetic Parameters for digoxin in urine:

CL_R: Renal clearance $Ae_{0-240h}/AUC_{0-240\,h}$

f_e: 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.

VASAVI INSTITUTIONAL ETHICS COMMITTEE APPROVED

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 C_{max} in any subject at any period, the data of such a subject will be excluded from the statistical analysis.

10 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.

10.1 Number of subjects

A total number of 28 healthy adult human subjects will be enrolled in the study. Additionally, 04 stand-by subjects will be included in order to dose 28 subjects in period I. As the same perpetrator (LANOXIN® Tablets USP 250 mcg) will be given to both the treatments and only the difference is going to be with and without spironolactone oral suspension, 28 subjects along with 04 stand-by subjects will be sufficient to provide a reliable estimate of the magnitude and variability of the interaction.

10.2 Analysis of variance

The log-transformed pharmacokinetic parameters (C_{max} and AUC_{0-96}) will be analyzed using Type III sum of squares, with the main effects of formulation, period, sequence and subjects nested within sequence as random effect. A separate ANOVA model will be used to analyze each of the parameters. The sequence effect will be tested at the 10% level of significance using the subjects nested within sequence mean square as the error term. Formulation and period effects will be tested at the 5% level of significance against the residual error (mean square error) from the ANOVA model as the error term. Each analysis of variance will include calculation of least-squares means, the difference between the adjusted formulation means and the standard error associated with the difference.

The above analyses will be done using the appropriate SAS® procedure.

VASAVI INSTITUTIONAL ETHICS COMMITTEE APPROVED

10.3 Two one-sided test for bioequivalence

Two one-sided test (Schuirmann's test, 1987) for bioequivalence and 90% confidence intervals for the ratio of least squares mean between drug formulations will be calculated, for ln-transformed data of C_{max} and AUC_{0-96}.

10.4 Power

The power of a test to detect 20% difference between treatment A & B will be computed and reported.

10.5 Ratio analysis

The comparison of interest is Treatment (A) Vs Treatment (B), so the ratios will be of the form: Treatment (A/B).

Ratio of least squares means of Treatment A & B will be computed for ln-transformed pharmacokinetic parameters C_{max} and AUC_{0-96}. Ratio analysis will be reported in percentage for ln-transformed pharmacokinetic parameters (C_{max} and AUC_{0-96}) for Digoxin.

10.6 Intra-Subject Variability

Intra-Subject variability will be computed for ln-transformed pharmacokinetic parameters (C_{max} and AUC_{0-96}) for Digoxin.

10.7 Criteria for evaluation

The presence or absence of DDI between two treatments (A and B) will be based on the statistical results of 90% confidence interval for the ratio of the geometric least squares mean for log-transformed pharmacokinetic parameters C_{max} and AUC_{0-96} for Digoxin.

Absence of drug-drug interaction will be established if the 90% CI for the ratio of population geometric means between two treatments (A and B), based on log-transformed data, present in the equivalence limits of 80.00 - 125.00% for C_{max} and AUC_{0-96}.

Summary statistics of pharmacokinetic profile of Digoxin (substrate drug) in the presence and absence of spironolactone (perpetrator) will be provided based on plasma and urine data. Renal clearance (CL_R)/ Percent Recovered and unchanged drug excreted in urine (f_e)/Amount Recovered for Digoxin will also be measured as supportive data.

VASAVI INSTITUTIONAL ETHICS COMMITTEE APPROVED

11 ASSESSMENT OF SAFETY

11.1 Specification of Safety Parameters

Vital signs (seated blood pressure, radial pulse rate and oral/aural temperature) will be measured as per section 11.2.

11.2 Methods and Timing for Assessing, Recording and Analyzing Safety Parameters

Clinical examination along with vital signs (seated blood pressure, radial pulse rate and oral/aural temperature) measurement will be carried out and recorded at check-in and at checkout of each period and/or at the termination of the study.

Clinical examination will be carried on prior to dosing on day 06 in each study period.

Oral/aural temperature will be measured prior to dosing.

Vital parameters – seated blood pressure and radial pulse rate will be measured at prior to dosing, 1.00, 3.00, 5.00 and 11.00 hours post dose in each period from Day 1 to Day 5, whereas from Day 6 to Day 9, vitals will be measured at prior to dosing, 1.00, 2.00, 3.00, 5.00, 8.00 and 11.00 hours post dose in each period. A window period of ± 45 minutes from the scheduled time point is allowed for post dose vitals recording.

ECG Measurements:

- ECG will be performed before period II check in and subject study eligibility will be assessed as per the principal investigator discretion.
- ECG will be performed at 1.00 and 3.00 hours post dose in each period on Day 6 with a window period of ± 45 minutes from schedule time.
- ECG will be performed at check-out of each period of the study.

Note:

- On Day 6 the Vital sings (i.e. up to 3.00 hours post dose) will be performed at bed side only.
- 2D echo will be performed at the time of screening.
- Dosing will be done under the supervision of cardiologist.
- For subjects who are randomized to treatment A, the vitals will be checked at 5.00 hours post dose during day 1 to Day 5. However, the vitals time points

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will be similar from day 6 for treatment groups A and B.

Vital signs may also be carried out at any time during the conduct of the study if the attending physician feels it necessary. In case of abnormality in vital signs during pre-dose vitals, recording, medical opinion will be taken whether to dose the subject or not.

Additional Laboratory evaluations (Biochemistry):

In each study period creatinine clearance and serum potassium levels to be measured on day 05 and the dosing eligibility of the subjects will be done as per the principal investigator discretion.

Risk evaluation and mitigation strategy:

Since healthy subjects are being enrolled in the study, there is no benefit to them. In general, the adverse reactions of digoxin are dose-dependent and occur at doses higher than those needed to achieve a therapeutic effect. Hence, adverse reactions are less common when digoxin is used within the recommended dose range or therapeutic serum concentration range and when there is careful attention to concurrent medications and conditions.

As Digoxin is a narrow therapeutic index drug, the drug administration will be done under the supervision of a cardiologist along with a continuous close monitoring by medical and para medical personnel. Since most of the digoxin is excreted unchanged in the urine, subjects with significant difference from normal serum creatinine or creatinine clearance values will not be considered for this study. In addition, only the subjects with normal 2D echo and QTc interval will be enrolled into the study.

Considering the single dose nature of this study and 28 days washout period between dosing periods, the effects caused by 250 mcg Tablet of Digoxin are not expected to impact on safety in the healthy volunteer population. Even if adverse events are observed, they are likely to be benign, transient, not require intervention. Nevertheless, close monitoring of blood pressure, pulse rate, and ECG will be conducted during the time maximum pharmacodynamic effects are observed (i.e. during 1 - 3 hours post dose).

Furthermore, subjects will be domiciled until day 04 (i.e. until 96 hours after drug

VASAVI INSTITUTIONAL ETHICS COMMITTEE APPROVED

administration in each period), thus providing an optimal setting for the clinical monitoring and, if necessary, management of any adverse events. However, subjects will only be allowed to leave the clinical center after clinical examination, ECG, and other safety measures are repeated at the end of study visit.

KCl Injection, KCl oral Syrup, Amiodarone Injection 150 mg, Xylocard Injection will be made available in the ICU at the time of study as a precautionary measure. Overall, based on the known AE profile of 250 mcg Tablet of Digoxin, strict screening, inclusion and exclusion criteria, routine safety assessments and planned close monitoring of known AE's, blood pressure, pulse rate, ECG, and 28 days washout period is considered sufficient to mitigate any potential risk to the subjects.

Laboratory evaluations performed during screening are as follows:

Blood tests:		Urine analysis
Hematology	Biochemistry	
Hemoglobin Total RBC Count Total WBC Count Platelet count Differential count Peripheral smear Blood group ESR	Random blood glucose Blood urea Serum creatinine Serum uric acid Serum sodium Serum potassium Serum chloride Serum calcium Serum magnesium	Physical Examination Chemical Examination Microscopic examination
Serology:	Liver Function Tests:	
HIV (1 & 2) antibodies HBsAg (Hepatitis Bsurface antigen) Anti-hepatitis C Virus (HCV) VDRL (RPR)	Total Bilirubin Direct Bilirubin SGOT (AST) SGPT (ALT) Alkaline phosphatase Total proteins Albumin Total Cholesterol	

Note: The laboratory parameters will be evaluated as per laboratory reference ranges (Appendix-IV). In rare instances, when the values are out of laboratory

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reference ranges, then those values will be correlated with clinical condition of the subject for further evaluation.

Laboratory (safety) evaluations performed at the end of study:

About 07 mL of blood will be collected from each subject for safety evaluation [which includes hematology (except blood group and Rh typing) and biochemistry (except random blood sugar) as per section 11.2] at the end of the study. If the subject does not come for the safety evaluation on scheduled time for any reason, follow-up will be done as per applicable Clinsync SOP.

11.3 Handling and reporting of Adverse Events and Serious Adverse Events

Definitions

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a medicinal (investigational) product whether or not related to the medicinal (investigational) product.

Adverse Drug Reaction (ADR): A response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of disease or for modification of physiological function.

Unexpected Adverse Drug Reaction: An adverse reaction, the nature or severity of which is not consistent with the applicable product information of Carospir® Spironolactone Oral Suspension, 25 mg/5 mL.

Serious Adverse Event (SAE): A serious adverse event (experience) or reaction is any untoward medical occurrence that at any dose

- Results in death,
- Is life-threatening,
- Requires inpatient hospitalization or causes prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity,
- Congenital anomaly/birth defect,

VASAVI INSTITUTIONAL ETHICS COMMITTEE APPROVED

- Requires intervention to prevent permanent impairment or damage

Note: The term "life threatening" in the definition of "serious adverse event" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it was more severe.

The causality assessment to the study treatment is characterized as:

TERM	DESCRIPTION	COMMENT
Certain/ Definite	A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary.	It is recognized that this stringent definition will lead to very few reports meeting the criteria, but this is useful because of the special value of such reports. It is considered that time relationships between drug administration and the onset and course of the adverse event are important in causality analysis. So also is the consideration of confounding features, but due weight must be placed on the known pharmacological and other characteristics of the drug product being considered. Sometimes the clinical phenomena described will also be sufficiently specific to allow a confident causality assessment in the absence of confounding features and with appropriate time relationships, e.g. penicillin anaphylaxis.
Probable/ Likely	A clinical event, including laboratory test abnormality, with	This definition has less stringent wording than for "certain" and does

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	a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.	not necessitate prior knowledge of drug characteristics or clinical adverse reaction phenomena. As stated no rechallenge information is needed, but confounding drug administration underlying disease must be absent.
Possible	A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.	This is the definition to be used when drug causality is one of other possible causes for the described clinical event.
Unlikely/ Remote	A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.	This definition is intended to be used when the exclusion of drug causality of a clinical event seems most plausible.
Conditional/ Unclassified	A clinical event, including laboratory test abnormality, reported as an adverse reaction, about which more data is essential for a proper assessment	

VASAVI INSTITUTIONAL ETHICS COMMITTEE APPROVED

	or the additional data are under examination.	
Unassessible/ Unclassifiable	A report suggesting an adverse reaction which cannot be judged because information is insufficient or contradictory, and which cannot be supplemented or verified.	

For each adverse event, the following information must be recorded in individual AE reporting forms:

- Type of adverse event
- Is it serious or non-serious?
- Date and time of onset
- Date and time of resolution
- Severity (mild, moderate or severe)
- Association with the study medication (remote, possibly related, probably related or definitely related)
- Action taken
- Outcome of adverse event (resolved or unresolved)
- Further details of the AE, if any.

Intensity of adverse events will be assessed as following:

Mild: An adverse event, Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated; generally, not interfering with normal activities.

Moderate: An adverse event, minimal, local or noninvasive intervention indicated; which is sufficiently discomforting to interfere with normal activities.

Severe: An adverse event, which is severe or medically significant but not immediately life threatening and does not cause hospitalization or prolongation of hospitalization; it incapacitates and prevents normal activities.

Subjects will be monitored throughout the study period for adverse events. Subjects will be instructed to bring to the notice of any study personnel of any

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adverse event that may occur during their stay at the clinical facility.

Subjects will also be specifically asked about any adverse events throughout the study period during the recording of vital signs. A medically qualified designate will be available round-the-clock during the period of housing at the clinical facility. The attending physician at the clinical facility will treat all the adverse events or serious adverse events. Any AE or SAE if required will be managed in-house in the well-equipped ICU, available at the study site by qualified physician and, if required subject will be transported in ambulance to the tertiary healthcare hospital for treatment, with which ClinSync has an agreement in place.

Recording of Adverse events

All adverse events including both observed and volunteered ones will be recorded on the appropriate AE form (As per applicable Clinsync SOP), irrespective of its association with the investigational products. Laboratory parameters, which are outside the clinically acceptable ranges but are not clinically significant as per PI discretion, must be recorded in the lab report and respective CRF page as Nil Significant. Clinically significant abnormal laboratory values that represent a change from baseline should be recorded diagnostically as AE's (e.g.: for elevated serum glucose, the AE would be described diagnostically as "hyperglycemia"). Determination of Clinical significance is based on the Investigator's medical judgment/discretion. PI shall evaluate the event for its severity, causality and seriousness that require immediate report to IEC and sponsor.

The institutional ethics committee (IEC), regulatory agency (ies), and the sponsor will be informed regarding the adverse events as necessary. Any serious adverse events will be recorded in appropriate Serious Adverse Event Reporting Form (As per applicable Clinsync SOP).

The investigator shall report all serious and unexpected adverse events to the licensing authority, the sponsor or his representative, whosever had obtained permission from the licensing authority for the conduct of the study and the ethics committee that accorded approval to the study protocol, within twenty-four hours of their occurrence. In case the Investigator fails to report any serious adverse event within the stipulated period, he shall have to furnish the reason for the delay

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to the satisfaction of the Licensing Authority along with the report of the serious adverse event. The report of the serious adverse event, after due analysis shall be forwarded by the investigator to the Licensing Authority, chairman of the Ethics Committee and the Head of the Institution where the trial has been conducted within fourteen calendar days of the occurrence of the serious adverse event.

Serious adverse events report submitted to Licensing Authority will be in color coded binding, where the reports of SAEs of Deaths are submitted in red cover, the report of SAEs of injury other than deaths in blue cover and the remaining cases of SAE report in white cover.

Each adverse event will be evaluated for duration, severity, and action taken, outcome and association with the study medication. The study may be suspended or terminated depending upon the seriousness of the adverse events.

Note: The reporting of all serious, unexpected suspected adverse reactions, any unexpected fatal or life-threatening suspected adverse reactions and any findings from the clinical study will be submitted as a per the timelines mentioned in 21 CFR 312.32 and 21 CFR 312.33 and compliance with the FDCA (21 U.S.C. §§ 301 et. seq.).

11.4 Type and Duration of the Follow-Up of Subjects

All adverse events will be followed until resolution or until the physician believes that there will be no further change. This may involve additional visits. Follow up will be continued as per applicable Clinsync SOP.

11.5 Criteria for the Termination of the Study

- Based on the safety of the subjects, the study can be suspended or terminated.
- The IEC/Sponsor/ regulatory can suspend/terminate the study any time during the monitoring due to one of the following reasons:
  a) Non-compliance to Protocol,
  b) Non-compliance to GCP
  c) Non-compliance to applicable regulatory guidelines.

12 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Direct access to source data/documents will be permitted to study-related monitoring, audits, IEC review, and regulatory inspection(s).

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13 QUALITY ASSURANCE

The raw data generated during the course of the study as well as reports will undergo quality assurance audit for conformance to this protocol and all the governing SOPs by auditors from the Quality Assurance Department of ClinSync Clinical Research Pvt. Ltd. The final report will contain a statement for quality assurance duly signed by auditors and the Head - Quality Assurance.

14 ETHICS

14.1 Institutional Ethics Committee

This protocol and corresponding informed consent form (ICD) (containing information about the study to be given to the subjects) to be used to obtain written informed consent of study subjects will be reviewed by the Institutional Ethics Committee (IEC) and subjects will not be enrolled into the study until the IEC approves the protocol and the ICD as submitted or with modification(s). The study will be conducted according to the requirement of the Declaration of Helsinki and ICH guidelines (Step 5) 'Guidance on Good Clinical Practice' and 'Guidance on Good Laboratory Practice'.

14.2 Written Informed Consent

Informed consent should be obtained before conducting any study-specific procedures (i.e. all of the procedures described in the protocol). The process of obtaining informed consent should be documented in the subject source documents.

The Principal Investigator or designated study personnel will inform the subjects (in a language understandable by the subject) before initiation of study through an oral presentation regarding the purpose, procedures to be carried out, investigational products, potential hazards and rights of the study subjects. The subjects will be required to understand and sign the ICD obtained prior to any study specific screening or other protocol required procedures being conducted. and one photocopy of the signed ICD will be given to each subject while the original will be filed in the trial master file.

If a subject is unable to read and write, an impartial witness will be present during the entire informed consent discussion. The informed consent form provided to all

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the subjects will be explained. The written consent will be obtained from all the subjects, who are able to read and understand. If any subject(s) is unable to read and understand the informed consent, his sign/thumb impression will be obtained along with the witness sign and date.

By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the subject and that informed consent was freely given by the subject.

14.3 Audio Video Consent

The Investigator/designee will safeguard the confidentiality of study data, which might lead to the identification of the individual subjects. Data of individual subjects can be disclosed only in a court of law under the orders of the presiding judge or in some cases may be required to communicate to Drug regulatory/Health authority.

In order to maintain the confidentiality, the videographer (person involved in recording the audio video consent) will be engaged as part of the study team. Prior to initiation of the study, the Investigator/study coordinator will define and allocate the activities of audio-video recording of informed consent process to the respective identified person as videographer.

Prior consent of the subject will be taken for audio-visual recording of informed consent process and the same will be documented by the Investigator. Such consent can be taken orally. Only those subjects who give the consent for the AV recording shall be included in the study.

In the present study Internet protocol camera will be used to record the Informed consent process in a closed room. The Internet protocol camera operator may not be physically present in the informed consent room. The recorded data will be archived for at least 05 years.

14.4 Subject Participation Fee

The subjects will be paid an adequate (IEC approved) participation fee for their participation in the study. In case of dropout/withdrawal of a subject before completion of the study, the subjects will be paid a pro-rated participation fee

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depending upon the extent of participation and any controversy pertaining to this will be forwarded to the IEC and the decision of the IEC will be final as well as binding on both the subjects and ClinSync Clinical Research Pvt. Ltd.

15 DATA HANDLING AND RECORD KEEPING

All data generated during the conduct of the study will be directly entered in the respective raw data forms. The computer-generated randomization schedule will also be treated as raw data. All raw data and transcribed data forms compiled by the study personnel involved in the study will be checked for completeness. All data related to the project will be in the custody of the Principal Investigator until transferred to archives.

15.1 Study Report and Documents

The final report format will be prepared as per ICH E3: Structure and Content of Clinical Study Reports and as per CDSCO regulatory requirements. Supplementary Documents such as screening records, Period I and Period II records etc. will be given along with study report. Frequency distributions of gender and race will be tabulated by treatment and sequence. Summary statistics for age, body weight, height, and Body Mass Index (BMI) will be tabulated by treatment sequence. Listings of pre study & post study laboratory values, listing of adverse event by subject, listing of urine drug scan by subject, listings of protocol deviations, summary of adverse events by organ class will be provided in clinical phase of CSR.

15.2 Archiving

All data generated in connection with this study, together with a copy of this protocol, signed ICDs and the final report will be archived for at least 05 years.

15.3 Confidentiality of Data

The data identifying each subject by name will be kept confidential and will be accessible only to the study personnel (involved in check-in procedure) and if necessary, to the QA auditors, IEC, sponsor's monitor and Regulatory agency (ies).

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16 FINANCING AND INSURANCE

ClinSync has agreement with "United India Insurance company Ltd.," for the insurance coverage of the volunteers participating in a Bioavailability study. They are covered by "Special Contingency Insurance Policy" for any exigency that may happen to them during the study.

17 PUBLICATION POLICY

Publication of results/findings of the study will be at the discretion of the sponsor. If published, the subject's identity will not be revealed.

18 REFERENCES

- NDA approval letter for Carospir® (Spironolactone) Oral Suspension, 25 mg/5 mL, NDA 209478
- Prescription information: Carospir® Spironolactone Oral Suspension, 25 mg/5 mL manufactured & distributed by CMP Pharma, Inc. 8026 US Highway 264A, Farmville, NC 27828.
- Prescription information: LANOXIN® (digoxin) Tablets, USP 250 mcg manufactured for Concordia Pharmaceuticals Inc., St. Michael, Barbados BB11005. Initial U.S. Approval: 1954; Revised: 12/2016
- USFDA Draft Guidance: Clinical Drug Interaction Studies - Study Design, Data Analysis, and Clinical Implications; October 2017
- International Conference on Harmonization; Guidance for Industry, E6 Good Clinical Practice: Consolidated Guidance.
- Declaration of Helsinki, 64th WMA General Assembly, Fortaleza, Brazil, October 2013.
- Good Clinical Practices For Clinical Research in India – Schedule Y.
- http://who-umc.org/Graphics/24734.pdf
- Guidance for Industry: Statistical Approaches to Establishing Bioequivalence, Center for Drug Evaluation and Research, January 2001
- Guidance for Industry - Bioavailability and Bioequivalence Studies for Orally Administered Drug Products - General Considerations / Guidance for Industry

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Conduct and Analysis of Bioavailability and Bioequivalence Studies (Part A: Oral Dosage Formulations Used for Systemic Effects).

- Advice/Information Request for spironolactone and digoxin IND 120929.

19 APPENDICES

Appendix I Schematic Diagram of Study Design

Appendix II Assessment Schedule

Appendix III Diet Schedule Form

Appendix-IV Laboratory Reference Ranges

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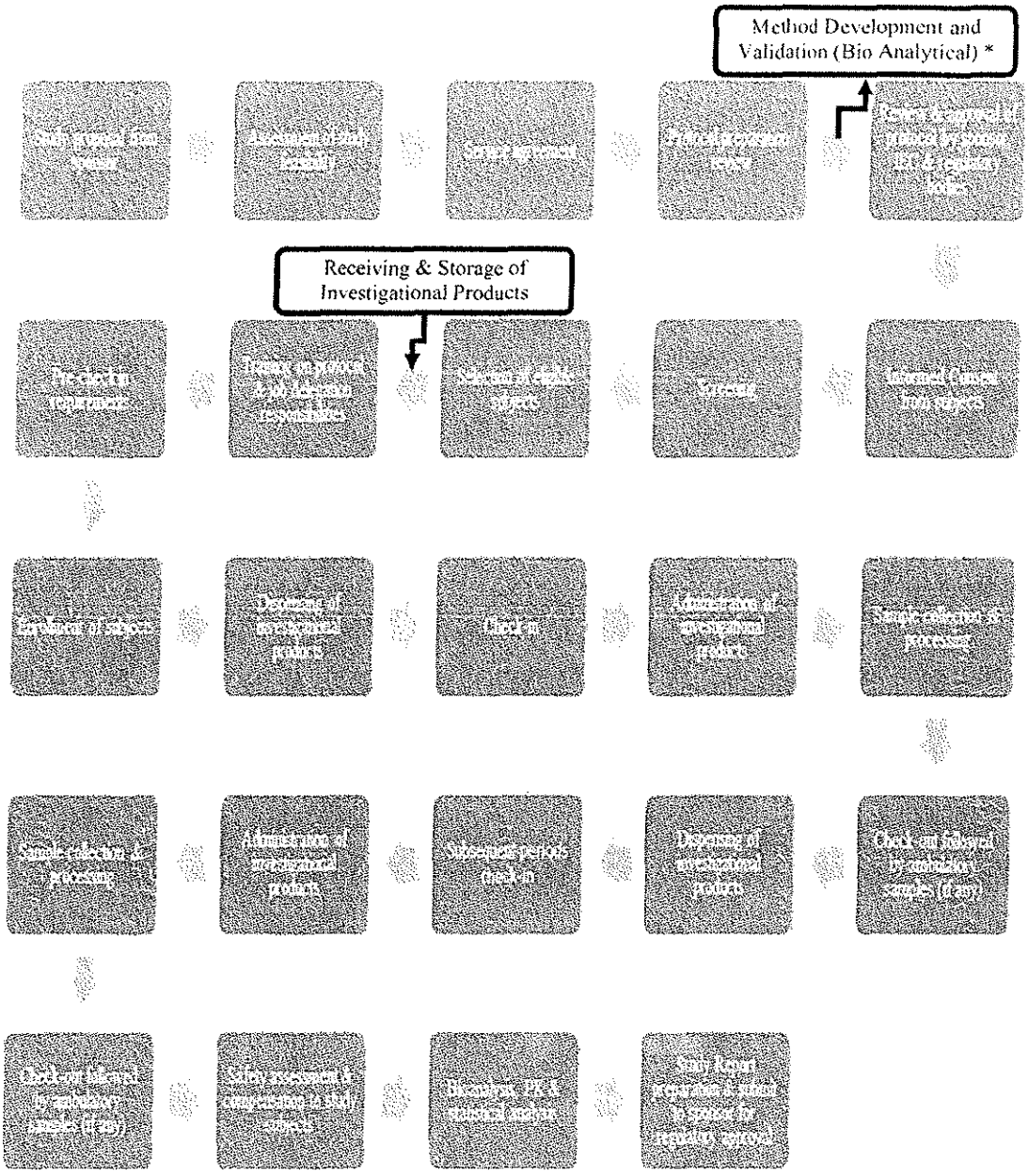
Appendix I: Schematic Diagram of Study Design



* Bioanalytical method development and validation will be completed before the start of the study

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Appendix II: Assessment schedule

The following time-and-events table illustrates the planned schedule of assessments:

	Screening ('-28' days to day '-3')	Period I and II										
		Day '-1'	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Informed consent	X----------X	-	-	-	-	-	-	-	-	-	-	-
Demography	X	-	-	-	-	-	-	-	-	-	-	-
Electrocardiogram	X	-	X[#]	-	-	-	-	X	-	-	-	X
2D Echocardiogram	X	-	-	-	-	-	-	-	-	-	-	-
Chest X-Ray* (P/A view)	X	-	-	-	-	-	-	-	-	-	-	-
Blood sample for Hematology	X	-	-	-	-	-	-	-	-	-	-	X[1]
Blood sample for Biochemistry	X	-	-	-	-	-	-	-	-	-	-	X[1]
Blood sample for serological examination	X	-	-	-	-	-	-	-	-	-	-	-
Urine analysis	X	-	-	-	-	-	-	-	-	-	-	-
Urine sample for drugs of abuse	-	X	-	-	-	-	-	-	-	-	-	-
Alcohol breath test	-	X	-	-	-	-	-	-	-	-	-	-
Pregnancy test	-	X[2]	-	-	-	-	-	-	-	-	-	-
Evaluation of inclusion/exclusion criteria[3]	X	X	-	-	-	-	-	-	-	-	-	-
Creatinine clearance and serum potassium levels	-	-	-	-	-	-	X	-	-	-	-	-
Dispensing[4]	-	X	X	X	X	X	X	X	X	X	X	-
Dosing	-	-	X[$]	X[$]	X[$]	X[$]	X[$]	X[$]	X[$]	X[$]	X[$]	-
Urine samples for Pharmacokinetics	-	-	-	-	-	-	-	X	X	X	X	X
Blood samples for Pharmacokinetics	-	-	-	-	-	-	-	X	X	X	X	X
Vital signs measurement	X	X	X	X	X	X	X	X	X	X	X	X
Diet schedule	-	X	X	X	X	X	X	X	X	X	X	-
Adverse Events monitoring	-	X	X	X	X	X	X	X	X	X	X	X

*Chest X-Ray (P/A view) will be taken for the volunteer whose X-Ray was taken more than 6 months prior to the dosing of period I.

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[4] ECG will be performed at 1.00 and 3.00 hours post dose in each period of the study and before check in of period-II & before checkout of each period.

$ Spironolactone Oral Suspension will be administered from Day 1 to Day 5 and Day 7 to Day 9, to the subjects who will be randomized to treatment B as per randomization scheme and treatment A or B will be administered on day 6.

The day prior to drug administration in each period for this protocol will be considered as Day '-1'.

The day of dosing in each period for this protocol will be considered as Day 1.

[1] Post study (hematology and biochemistry) laboratory evaluations will be done at last blood sample collection of the study.

[2] Serum pregnancy test will be done for female subjects with child bearing potential and Urine pregnancy test will be done for post-menopausal female subjects during screening, each period check-in (Day '-1').

[3] Evaluation of inclusion/exclusion criteria will be carried out prior to check-in of period I of the study.

[4] Investigational products (substrate) will be dispensed a day before dosing and perpetrator will be dispensed on the day of dosing in each period of the study.

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Appendix III: Diet Schedule Form

Day 01

Name Of Cooked Food		Wt. In (g)	Proteins (g)	Carbohydrates (g)	Fats (g)	K.Cal
Dinner (Day -01)	Plain Rice	500	11	125	0.75	550.75
	Pulka (1)	35	03	17.5	0.45	86.05
	Red Gram Dhal	100	4.4	12.5	1.4	80.20
	Ladies Finger Curry	100	3.5	13	6.0	120.00
	Chutney	30	0.3	1.3	1.6	20.80
	Sambar	150	3.7	11	3.5	90.30
	Curd	100	3.1	3.0	4.0	60.40
	Papad (1)	10	1.2	0.8	1.5	21.50
						Total = 1030.00
Breakfast **0.50** hour after dosing	Puri	75	6.1	35.0	8.4	240.00
	Potato Curry	100	1.5	16.0	6.0	124.00
						Total =364.00
Lunch 04.00 hour after dosing	Plain Rice	500	11	125	0.75	550.75
	Pulka (1)	35	03	17.5	0.45	86.05
	Red Gram Dhal	100	4.4	12.5	1.4	80.20
	Capsicum Curry	100	1.00	12.00	6.00	106.00
	Chutney	30	0.3	1.3	1.6	20.80
	Sambar	100	2.5	7	2.5	60.50
	Curd	100	3.1	3.0	4.0	60.40
	Papad (1)	10	1.2	0.8	1.5	21.50
						Total = 986.20
Snacks 08.00 hour after dosing	Onion Pakori	60	6.6	25.0	12.8	241.60
						Total = 241.60
Dinner 12.00 At least 10 hours before dosing	Plain Rice	500	11	125	0.75	550.75
	Pulka (1)	35	03	17.5	0.45	86.05
	Red Gram Dhal	100	4.4	12.5	1.4	80.20
	Egg Curry	100	6.0	7.0	7.0	115.00
	Chutney	30	0.3	1.3	1.6	20.80
	Sambar	100	2.5	7	2.5	60.50
	Curd	100	3.1	3.0	4.0	60.40
	Papad (1)	10	1.2	0.8	1.5	21.50
	Total					**995.20**
						Grand Total = 2587.00

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Day 02

Name Of Cooked Food		Wt. In (g)	Proteins (g)	Carbohydrates (g)	Fats (g)	K.Cal
Breakfast 0.50 hour after dosing	Idly (4)	160	6.5	45.5	0.4	211.6
	Sambar	100	2.5	7	2.5	60.50
	Chutney (Coconut)	80	3.0	9.0	15	179.00
						Total = 451.10
Lunch 04.00 hour after dosing	Plain Rice	500	11	125	0.75	550.75
	Pulka (1)	35	03	17.5	0.455	86.05
	Red Gram Dhal	100	4.4	12.5	1.4	80.20
	Cluster beans curry	125	4.0	10.0	6.0	110.00
	Chutney	30	0.3	1.3	1.6	20.80
	Sambar	100	2.5	7	2.5	60.50
	Curd	100	3.1	3.0	4.0	60.40
	Papad (1)	10	1.2	0.8	1.5	21.50
						Total = 990.20
Snacks 08.00 hour after dosing	Samosa (1)	65	2.5	21	12.5	206.50
	Plain Rice	500	11	125	0.75	550.75
	Pulka (1)	35	03	17.5	0.45	86.05
	Red Gram Dhal	100	4.4	12.5	1.4	80.20
	Ridge Gourd curry	100	1.0	4.0	5.0	65.00
	Chutney	30	0.3	1.3	1.6	20.80
	Tomato Rasam	100	1.0	4.0	2.0	38.00
	Curd	100	3.1	3.0	4.0	60.40
Dinner 12.00 At least 10 hours before dosing	Papad (1)	10	1.2	0.8	1.5	21.50
						Total = 922.70
						Grand Total = 2570.50

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Day 03

Name Of Cooked Food		Wt. In (g)	Proteins (g)	Carbohydrates (g)	Fats (g)	K.Cal
Breakfast 0.50 hour after dosing	Vada (4)	88	10.5	30	11.5	265.50
	Sambar	75	1.75	4.75	2.00	44.00
	Chutney (Coconut)	80	3.00	9.00	15.0	179.00
						Total = 488.50
Lunch 04.00 hour after dosing	Plain Rice	500	11	125	0.75	550.75
	Pulka(1)	35	03	17.5	0.455	86.05
	Red Gram Dhal	100	4.4	12.5	1.4	80.20
	Nutrella curry	100	5.00	10.0	6.00	114.00
	Chutney	30	0.3	1.3	1.6	20.80
	Sambar	100	2.5	7	2.5	60.50
	Curd	100	3.1	3.0	4.0	60.40
	Papad (1)	10	1.2	0.8	1.5	21.50
						Total = 994.20
Snacks 08.00 hour after dosing	Masala Vada (Gare)	60	6.5	20.0	7.0	169.00
						Total = 169.00
Dinner 12.00 At least 10 hours before dosing	Plain Rice	500	11	125	0.75	550.75
	Pulka(1)	35	03	17.5	0.45	86.05
	Red Gram Dhal	100	4.4	12.5	1.4	80.20
	Brinzal Curry	100	1.00	4.00	5.00	65.00
	Chutney	30	0.3	1.3	1.6	20.80
	Tomato Rasam	100	1.0	4.0	2.0	38.00
	Curd	100	3.1	3.0	4.0	60.40
	Papad (1)	10	1.2	0.8	1.5	21.50
						Total = 922.70
						Grand Total = 2574.40

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Day 04

Name Of Cooked Food		Wt. In (g)	Proteins (g)	Carbohydrates (g)	Fats (g)	K.Cal
Breakfast **0.50** hour after dosing	Vermicelli Upma	240	8.7	63.0	12.00	390.0
	Tomato Chutney	60	0.6	2.6	3.2	41.60
						Total = 431.60
Lunch 04.00 hours after dosing	Plain Rice	500	11	125	0.75	550.75
	Pulka(1)	35	03	17.5	0.455	86.05
	Red Gram Dhal	100	4.4	12.5	1.4	80.20
	Kovi (Donda) curry	100	1.6	6.0	5.6	78.00
	Chutney	30	0.3	1.3	1.6	20.80
	Sambar	100	2.5	7	2.5	60.50
	Curd	100	3.1	3.0	4.0	60.40
	Papad (1)	10	1.2	0.8	1.5	21.50
						Total = 958.20
Snacks 08.00 hours after dosing	Sandwiches (2)	65	3.2	14.0	14.1	194.00
						Total = 194.00
Dinner 12.00 At least 10 hours before dosing	Plain Rice	500	11	125	0.75	550.75
	Pulka(1)	35	03	17.5	0.45	86.05
	Red Gram Dhal	100	4.4	12.5	1.4	80.20
	Masala Brinzal	100	3.0	7.0	10.0	130.0
	Chutney	30	0.3	1.3	1.6	20.80
	Tomato Rasam	100	1.0	4.0	2.0	38.00
	Curd	100	3.1	3.0	4.0	60.40
	Papad (1)	10	1.2	0.8	1.5	21.50
						Total = 987.70
						Grand Total = 2571.50

VASAVI INSTITUTIONAL ETHICS COMMITTEE APPROVED

Day 05

Name Of Cooked Food		Wt. In (g)	Proteins (g)	Carbohydrates (g)	Fats (g)	K.Cal
Breakfast 0.50 hour after dosing	Masala Dosa	100	3.8	30	6.5	192.00
	Chutney (Coconut)	80	3.00	9.00	15.0	179.00
						Total = 371.00
Lunch 04.00 hours after dosing	Plain Rice	500	11	125	0.75	550.75
	Pulka(1)	35	03	17.5	0.455	86.05
	Red Gram Dhal	100	4.4	12.5	1.4	80.20
	Mixed Veg curry	100	5.0	13	6.0	126.00
	Chutney	30	0.3	1.3	1.6	20.80
	Sambar	100	2.5	7	2.5	60.50
	Curd	100	3.1	3.0	4.0	60.40
	Papad (1)	10	1.2	0.8	1.5	21.50
						Total = 1006.20
Snacks 08.00 hours after dosing	Double ka Meetha	105	4.1	24	17.7	276.00
						Total = 276.00
Dinner 12.00 At least 10 hours before dosing	Plain Rice	500	11	125	0.75	550.75
	Pulka(1)	35	03	17.5	0.45	86.05
	Red Gram Dhal	100	4.4	12.5	1.4	80.20
	Ladies Finger Curry	100	3.5	13	6.0	120.00
	Chutney	30	0.3	1.3	1.6	20.80
	Tomato Rasam	100	1.0	4.0	2.0	38.00
	Curd	100	3.1	3.0	4.0	60.40
	Papad (1)	10	1.2	0.8	1.5	21.50
						Total = 977.70
						Grand Total = 2630.90

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Day 06

Name Of Cooked Food		Wt. In (g)	Proteins (g)	Carbohydrates (g)	Fats (g)	K.Cal
Lunch 04.00 hours after dosing	Plain Rice	500	11	125	0.75	550.75
	Pulka(1)	35	03	17.5	0.455	86.05
	Red Gram Dhal	100	4.4	12.5	1.4	80.20
	Potato Curry	100	1.5	16	6.0	124.00
	Chutney	30	0.3	1.3	1.6	20.80
	Sambar	150	3.7	11	3.5	90.30
	Curd	100	3.1	3.0	4.0	60.40
	Papad (1)	10	1.2	0.8	1.5	21.50
						Total = 1034.00
Snacks 08.00 hours after dosing	Milk with 10g sugar	200ml	7.0	20.0	6.0	162.00
	Bread	40	3.8	25.8	0.9	126.50
						Total = 288.50
Dinner 12.00 hours after dosing	Plain Rice	500	11	125	0.75	550.75
	Pulka(1)	35	03	17.5	0.45	86.05
	Red Gram Dhal	100	4.4	12.5	1.4	80.20
	Brinzal Curry	100	1.00	4.00	5.00	65.00
	Chutney	30	0.3	1.3	1.6	20.80
	Sambar	150	3.7	11	3.5	90.30
	Curd	100	3.1	3.0	4.0	60.40
	Papad (1)	10	1.2	0.8	1.5	21.50
						Total = 975.00
						Grand Total = 2297.50

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Day 07

Name Of Cooked Food		Wt. In (g)	Proteins (g)	Carbohydrates (g)	Fats (g)	K.Cal
Breakfast 0.50 hour after dosing	Idly (4)	160	6.5	45.5	0.4	211.6
	Sambar	100	2.5	7	2.5	60.50
	Chutney (Coconut)	80	3.0	9.0	15	179.00
						Total = 451.10
Lunch 04.00 hours after dosing	Plain Rice	500	11	125	0.75	550.75
	Pulka(1)	35	03	17.5	0.455	86.05
	Red Gram Dhal	100	4.4	12.5	1.4	80.20
	Tomato Methi Curry	100	5.00	10.0	5.00	68.00
	Chutney	30	0.3	1.3	1.6	20.80
	Sambar	100	2.5	7	2.5	60.50
	Curd	100	3.1	3.0	4.0	60.40
	Papad (1)	10	1.2	0.8	1.5	21.50
						Total = 948.20
Snacks 08.00 hours after dosing	Masala Vada (Gare)	60	6.5	20.0	7.0	169.00
						Total = 169.00
Dinner 12.00 hours after dosing	Plain Rice	500	11	125	0.75	550.75
	Pulka(1)	35	03	17.5	0.45	86.05
	Red Gram Dhal	100	4.4	12.5	1.4	80.20
	Egg Curry	100	6.0	7.0	7.0	115.00
	Chutney	30	0.3	1.3	1.6	20.80
	Sambar	100	2.5	7	2.5	60.50
	Curd	100	3.1	3.0	4.0	60.40
	Papad (1)	10	1.2	0.8	1.5	21.50
						Total = 995.20
						Grand Total = 2563.50

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Day 08

Name Of Cooked Food		Wt. In (g)	Proteins (g)	Carbohydrates (g)	Fats (g)	K.Cal
Breakfast 0.50 hour after dosing	Vada (4)	88	10.5	30	11.5	265.50
	Sambar	75	1.75	4.75	2.00	44.00
	Chutney (Coconut)	80	3.00	9.00	15.0	179.00
						Total = 488.50
Lunch 04.00 hours after dosing	Plain Rice	500	11	125	0.75	550.75
	Pulka(1)	35	03	17.5	0.455	86.05
	Red Gram Dhal	100	4.4	12.5	1.4	80.20
	Beans curry	100	4.0	08	5.0	93.00
	Chutney	30	0.3	1.3	1.6	20.80
	Sambar	100	2.5	7	2.5	60.50
	Curd	100	3.1	3.0	4.0	60.40
	Papad (1)	10	1.2	0.8	1.5	21.50
						Total = 973.20
Snacks 08.00 hours after dosing	Punugulu	60	4.5	16.0	8.0	154.00
	Tomato Chutney	60	0.6	2.6	3.2	41.60
						Total = 195.60
Dinner 12.00 hours after dosing	Plain Rice	500	11	125	0.75	550.75
	Pulka(1)	35	03	17.5	0.45	86.05
	Red Gram Dhal	100	4.4	12.5	1.4	80.20
	Capsicum Curry	100	1.00	12.00	6.00	106.00
	Chutney	30	0.3	1.3	1.6	20.80
	Tomato Rasam	100	1.0	4.0	2.0	38.00
	Curd	100	3.1	3.0	4.0	60.40
	Papad (1)	10	1.2	0.8	1.5	21.50
						Total = 963.70
						Grand Total = 2621.00

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Day 09

Name Of Cooked Food		Wt. In (g)	Proteins (g)	Carbohydrates (g)	Fats (g)	K.Cal
Breakfast 0.50 hour after dosing	Chapathi	75	6.5	37.0	3.5	205.50
	Potato Curry	100	1.5	16	6.0	124.00
						Total = 329.50
Lunch 04.00 hours after dosing	Plain Rice	500	11	125	0.75	550.75
	Pulka(1)	35	03	17.5	0.45	86.05
	Red Gram Dhal	100	4.4	12.5	1.4	80.20
	Carrot Peas curry	100	3.0	10.0	6.0	106.00
	Chutney	30	0.3	1.3	1.6	20.80
	Sambar	100	2.5	7	2.5	60.50
	Curd	100	3.1	3.0	4.0	60.40
	Papad (1)	10	1.2	0.8	1.5	21.50
						Total = 986.20
Snacks 08.00 hours after dosing	Samosa(1)	65	2.5	21.0	12.5	206.50
						Total = 206.50
Dinner 12.00 hours after dosing	Veg pulavo	600	14	114	15	647.00
	Mirch ka Salan(Capsicum)	190	3	18	15.4	222.60
	Sambar	100	2.5	7	2.2	60.50
	Curd	100	3.1	3.0	4.0	60.40
						Total = 990.50
						Grand Total = 2512.70

Note: 1g PROTIEN = 4 K.Cal; 1g CHO = 4 K.Cal; 1g FAT = 9 K.Cal

References: Nutritive Value of Indian Foods by Gopalan et.al, NIN, Count What You Eat by Swaran Pasricha, NIN and Food Composition Table of Indian foods 2017, NIN.

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Appendix-IV: Laboratory Reference Ranges

PARAMETER	LABORATORY REFERENCE RANGES
HEMATOLOGY	
Hemoglobin	Male:13-18 g/dL Female:11.5-16 g/dL
WBC Count	4000 – 11000 Cell/cumm
RBC Count	4.5- 5.5 mil/µl
Platelet Count	1.50-4.50 lakhs/cumm
Neutrophils	40-75 %
Lymphocytes	20-40 %
Eosinophils	01-06 %
Monocytes	02-10 %
Basophils	00-01 %
ESR	0-15 mm/hr
CLINICAL BIOCHEMISTRY	
Bilirubin Total	<1.0 mg/dL
Bilirubin Direct	<0.3 mg/dL
Alkaline Phosphatase	50-136 U/L
AST/SGOT	15-37 U/L
ALT/SGPT	30-65 U/L
Protein Total	6.4-8.2 g/dL
Albumin	3.4-5.0 g/dL
Urea	10-50 mg/dL

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PARAMETER	LABORATORY REFERENCE RANGES
Creatinine	0.8-1.3 mg/dL
Uric Acid	3.5-7.2 mg/dL
Glucose Random	70-130 mg/dL
Total Cholesterol	<200 mg/dL
Creatinine clearance	80-120 ml/min
Sodium	135-145 mmol/L
Potassium	3.5-5.5 mmol/L
Chloride	94-108 mmol/L
Calcium	8.5-10.1 mg/dL
CLINICAL PATHOLOGY	
Reaction (pH)	5.0-8.0
Specific Gravity	1.000-1.030
Proteins	Nil
Glucose	Nil
Ketones	Negative
Bilirubin	Negative
Blood	Negative
Urobilinogen	Normal
PUS Cells (WBC)	0-5/HPF
Urine RBC	Nil
U.Epithelial Cells	0-5/HPF

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PARAMETER	LABORATORY REFERENCE RANGES
Casts and Crystals	Nil
IMMUNOLOGY/SEROLOGY	
Hepatitis B Surface Antigen (HbsAg)	<1.0-Negative >1.0-Positive
Hepatitis C Virus (HCV)	<1.0-Negative >1.0-Positive
HIV 1 & 2 Antibody	<1.0-Negative >1.0-Positive
VDRL	1:8 & Above Significant

Note: Referral diagnostic ranges will be considered for the parameters which are not covered in the above table.

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PROTOCOL AMENDMENT

Protocol No.: C-CMP-SPI-001

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

Protocol No.: C-CMP-SPI-001; Version:01; Date: 13 Feb 2018; Version:02; Date: 07 Aug 2018; Amendment No.: 01; Date: 19 Dec 2018;

Section	Present statement in Protocol	Changed to present statement in Protocol	Reason
Protocol title page	**CRO & Clinical Study site** ClinSync Clinical Research Pvt. Ltd. #4-1-1, Hayathnagar, Hyderabad, Telangana – 501505, India.	**CRO & Clinical Study site** ClinSync Clinical Research Pvt. Ltd. JSR Mall, Plot No.7 to 18, Madinaguda Village, Serilingampally Mandal, Hyderabad-500050, Telangana, India.	Administrative change
Section 1: Study Information	**Principal Investigator** Dr. Roopali K. Somani, MD Pharmacology Clinical Pharmacology Department, ClinSync Clinical Research Pvt. Ltd., #4-1-1, Hayathnagar, Hyderabad, Telangana - 501505, India. Phone No.: +91-40-24203001/2/3 Fax No.: +91-40-24203000 Email ID: roopali.somani@clinsynccro.com.	**Principal Investigator** Dr. P. Venkatesh, MBBS, Ph.D Clinical Pharmacology Department, ClinSync Clinical Research Pvt. Ltd., JSR Mall, Plot No.7 to 18, Madinaguda Village, Serilingampally Mandal, Hyderabad-500050, Telangana, India. Phone No.: +91-40-24203001/2/3 Fax No.: +91-40-24203000 Email ID: venkatesh.p@clinsynccro.com	
	Sub Investigator Dr. Sagar Chandra S Bhuyar, MD General Medicine, DNB (cardiology)	**Sub Investigator** Dr. Sagar Chandra S Bhuyar, MD General Medicine, DNB (cardiology)	

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	ClinSync Clinical Research Pvt .Ltd. #4-1-1, Hayathnagar, Hyderabad, Telangana - 501505, India. Phone No.: +91-40-24203001/2/3 Fax No.: +91-40-24203000	ClinSync Clinical Research Pvt. Ltd., JSR Mall, Plot No.7 to 18, Madinaguda Village, Serilingampally Mandal, Hyderabad-500050, Telangana, India. Phone No.: +91-40-24203001/2/3 Fax No.: +91-40-24203000.	
	Clinical Investigator's Dr. Chandra Prakash Vijaya Ram, MBBS Clinical Pharmacology Department, ClinSync Clinical Research Pvt. Ltd., #4-1-1, Hayathnagar, Hyderabad, Telangana - 501505, India. Phone No.: +91-40-24203001/2/3 Fax No.: +91-40-24203000 Email ID: cp.researchscientist@clinsyncclro.com Dr. Vani Malisetti, MD OBG Clinical Pharmacology Department, ClinSync Clinical Research Pvt. Ltd., #4-1-1, Hayathnagar, Hyderabad, Telangana - 501505, India. Phone No.: +91-40–24203001/2/3 Fax No.: +91-40-24203000 Email ID: vani.malisetti@clinsyncclro.com Dr. Polepally Praveen Kumar,	**Clinical Investigator** Dr. Chandra Prakash Vijaya Ram, MBBS Clinical Pharmacology Department, ClinSync Clinical Research Pvt. Ltd., JSR Mall, Plot No.7 to 18, Madinaguda Village, Serilingampally Mandal, Hyderabad-500050, Telangana, India. Phone No.: +91-40-24203001/2/3 Fax No.: +91-40-24203000 Email ID: cp.researchscientist@clinsyncclro.com	

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	PK Investigator Dr. Jaganmohan S, M.Pharm, Ph. D, PDF PK, BS and MW Department, ClinSync Clinical Research Pvt. Ltd. #4-1-1, Hayathnagar, Hyderabad, Telangana - 501505, India. Phone No.: +91-40-24203001/2/3; Ext: 604 Fax No.: +91-40-24203000	**PK Investigator** Dr. Jaganmohan S, M.Pharm, Ph.D, PDF PK, BS and MW Department, ClinSync Clinical Research Pvt. Ltd., JSR Mall, Plot No.7 to 18, Madinaguda Village, Serilingampally Mandal, Hyderabad-500050, Telangana, India. Phone No.: +91-40-24203001/2/3;	

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	Email ID: jaganmohan.somagoni@clinsynccro.com.	Ext: 301 Fax No.: +91-40-24203000 Email ID: jaganmohan.somagoni@clinsynccro.com.	
	Statistical Investigator Mr. Tushar Dilip Shinde, M. Sc BS Department, ClinSync Clinical Research Pvt. Ltd., #4-1-1, Hayathnagar, Hyderabad, Telangana - 501505, India. Phone No.: +91-40-24203001/2/3; Ext: 604 Fax No.: +91-40-24203000 Email ID: tushar.shinde@clinsynccro.com	**Statistical Investigator** Mr. Tushar Dilip Shinde, M. Sc BS Department, ClinSync Clinical Research Pvt. Ltd., JSR Mall, Plot No.7 to 18, Madinaguda Village, Serilingampally Mandal, Hyderabad-500050, Telangana, India. Phone No.: +91-40-24203001/2/3; Ext: 301 Fax No.: +91-40-24203000 Email ID: tushar.shinde@clinsynccro.com	
	Clinical Facility and Screening Facility ClinSync Clinical Research Pvt. Ltd. Clinical Pharmacology Department, #4-1-1, Hayathnagar, Hyderabad, Telangana - 501505, India. Phone no.: +91-40 – 24203001/2/3 Fax no.: +91-40-24203000.	**Clinical Facility and Screening Facility** ClinSync Clinical Research Pvt. Ltd. Clinical Pharmacology Department, JSR Mall, Plot No.7 to 18, Madinaguda Village, Serilingampally Mandal, Hyderabad-500050, Telangana, India. Phone no.: +91-40-24203001/2/3 Fax no.: +91-40-24203000.	

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	Bioanalytical, Pharmacokinetic and Statistical Facility ClinSync Clinical Research Pvt. Ltd., #4-1-1, Hayathnagar, Hyderabad, Telangana - 501505, India. Phone no.: +91-40 – 24203001/2/3 Fax no.: +91-40-24203000.	**Bioanalytical, Pharmacokinetic and Statistical Facility** ClinSync Clinical Research Pvt. Ltd. Clinical Pharmacology Department, JSR Mall, Plot No.7 to 18, Madinaguda Village, Serilingampally Mandal, Hyderabad-500050, Telangana, India. Phone no.: +91-40-24203001/2/3 Fax no.: +91-40-24203000.	
	X-Ray Facility SUNRISE HOSPITALS H. #: 4-9-321, Plot # 4 & 7, Hayathnagar, R.R. Dist, Hyderabad-501505. Phone no.: 9700009432 And/or ClinSync Clinical Research Pvt. Ltd. Clinical Pharmacology Department, #4-1-1,Hayathnagar,Hyderabad, Telangana-501505, India.	**X-Ray Facility** Srikara Hospitals #222&223, Mythri Nagar,Phase-II, Madinaguda, Miyapur, Hyderabad-500049, Ph: +91 40 -47470000, + 91 772 999 0002. And/or ClinSync Clinical Research Pvt. Ltd. Clinical Pharmacology Department, JSR Mall, Plot No.7 to 18, Madinaguda Village, Serilingampally Mandal, Hyderabad-500050, Telangana, India. Phone no.: +91-40-24203001/2/3 Fax no.: +91-40-24203000.	

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	Emergency Services SUNRISE HOSPITALS H. #: 4-9-321, Plot # 4 & 7, Hayathnagar, R.R. Dist, Hyderabad-501505. Phone no.: 9700009432	**Emergency Services** Srikara Hospitals #222&223, Mythri Nagar, Phase-II, Madinaguda, Miyapur, Hyderabad-500049, Ph: +91 40 -47470000, + 91 772 999 0002	
6.4 Sampling Procedure	The plasma samples will be then stored upright in a box containing dry ice or in a freezer at a temperature of -20 ± 5°C for interim storage (pre-dose and post-dose samples up to 4.00 hours) and then will be transferred to -70 ± 15°C at the clinical site.	The plasma samples will be stored in a freezer of CCM department at -70 ± 10°C.	Change in storage temperature due to revision of the SOP

Note: The above said changes are applicable throughout the protocol and related documents.

Prepared By (MW): 19 Dec 2018
(Sign & date)

Reviewed By (QA): Ashok 19 Dec 2018
(Sign & date)

Approved By (PI/CI): 19 Dec 2018
(Sign & date)

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PROTOCOL AMENDMENT

Protocol No.: C-CMP-SPI-001

I, on behalf of CMP Development LLC, USA, have read, understood and approve this Protocol Amendment No.01; Dated on 19 Dec 2018 for conducting the study at ClinSync Clinical Research Pvt. Ltd as per their Procedures.

Study Protocol No : C-CMP-SPI-001; Version:01; Date: 13 Feb 2018
Version:02; Date: 07 Aug 2018

Amendment No : 01; Dated on 19 Dec 2018

Date: 12/20/18

Authorized signatory

Gerald Sakowski

Chief Executive Officer

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16.1.2 SAMPLE CASE REPORT FORM