scPharmaceuticals

CCS Cardiovascular Clinical Sciences

STATISTICAL ANALYSIS PLAN (SAP)

Open-label, Single-dose, Randomized, Two-way (two-period) Crossover Study to Compare the Pharmacokinetics and Pharmacodynamics of SCP-111 (furosemide injection) Administered as a Subcutaneous Injection vs Furosemide Administered as an Intravenous Injection in Healthy Volunteers

IND Number:	158047
Protocol Number:	scP-04-001
Study Type:	Phase I
Investigational Drug-Device Combination:	SCP-111 Autoinjector
SAP	v2.0 15Jul2024

SUMMARY OF CHANGES

Version	Date	Affected Section(s)	Change	Reason for Change
Original	15Jun2024	N/A	Original Version	New Document
Amendment 1	15Jul2024	Throughout	Removed analyses by treatment sequence	Analysis not required; treatment sequence effect not expected
Amendment 1	15Jul2024	Section 9.3.3	Removed t-test for within and between group comparisons for injection site pain	Differences not expected and analyses to remain descriptive
Amendment 1	15Jul2024	Section 9.3.4.1	Added calculation for eGFR	eGFR analyses to be included in clinical chemistry summaries
Amendment 1	15Jul2024	Section 9.3.4.2	Updated analyses for vital signs	Vital signs to be summarized as defined
Amendment 1	15Jul2024	Section 9.3.5.3	Updated definitions for prior and concomitant medications	Prior and concomitant medications to be summarized as defined
Amendment 1	15Jul2024	Section 9.3.5.4	Protocol deviations to be listed by date, not timepoint	Protocol Deviations to be summarized as defined

APPROVAL PAGE

Protocol Name: scP-04-001

Study Title: Open-label, Single-dose, Randomized, Two-way (two-period) Crossover Study to Compare the Pharmacokinetics and Pharmacodynamics of SCP-111 (furosemide injection) Administered as a Subcutaneous Injection vs Furosemide Administered as an Intravenous Injection in Healthy Volunteers

SAP Version Date: 15Jul2024

Version Number: 2.0

The undersigned have reviewed this analysis plan and approve of it in its entirety.

Signature **Date**

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ABBREVIATIONS

AE	Adverse Event
AUC	Area Under the Curve
AUC_{ext}	Percentage of the AUC that is extrapolated beyond the last measurable concentration
AUC_{inf}	Area Under the Curve to Infinity
AUC_{last}	Area Under the Curve to Last Quantifiable Concentration
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
C	Celsius
CL	Systemic Clearance
CL/F	Apparent Systemic Clearance
C_{max}	Maximum Plasma Concentration
CRF	Case Report Form
CRU	Clinical Research Unit
CV	Cardiovascular
Dosing (Group)	A set of subjects receiving their first and second doses of the study drug on the same dates. This grouping accounts for potential timing differences in drug administration in the statistical model to ensure accurate analysis of treatment effects.
ECG	Electrocardiogram
eGFR	Estimated Glomerular Filtration Rate
F	Bioavailability
IV	Intravenous
K Excretion	Urinary Potassium Excretion
MedDRA	Medical Dictionary for Regulatory Activities
Na Excretion	Urinary Sodium Excretion
NYHA	New York Heart Association
PD	Pharmacodynamics
Period	A specific phase in the crossover study during which a subject receives one of the treatments. Each period is followed by a washout

	phase before the next treatment is administered. For example, Period 1 is the time frame for the first treatment, and Period 2 is the time frame for the second treatment.
PK	Pharmacokinetics
SAE	Serious Adverse Event
SC	Subcutaneous
Sequence	The order in which a subject receives different treatments during the crossover study. For example, Sequence 1 might involve receiving IV furosemide first followed by SC SCP-111, while Sequence 2 might involve the reverse order.
T_{max}	Time to Maximum Concentration
λ_z	Terminal Elimination Rate Constant
$t_{½}$	Terminal Half-Life
UO	Urine Output
$V_{z/F}$	Apparent Systemic Volume of Distribution
V	Systemic Volume of Distribution

1. TITLE

Open-label, Single-dose, Randomized, Two-way (two-period) Crossover Study to Compare the Pharmacokinetics and Pharmacodynamics of SCP-111 (furosemide injection) Administered as a Subcutaneous Injection vs Furosemide Administered as an Intravenous Injection in Healthy Volunteers.

2. STUDY DESIGN

This is an open-label, single-center, single-dose, randomized, two-way (two-period) crossover study in healthy volunteers. Each Subject will complete Screening, Baseline, Treatment (two cross-over periods), and Follow-up Phases.

The Screening Phase will be conducted on an outpatient basis between 14 and 3 days prior to the Baseline visit. Subjects will be instructed to maintain a < 2-gram sodium diet and abstain from caffeine-containing products within 2 days prior to admission to the Clinical Research Unit (CRU). On arrival to the CRU (Day 0), baseline and final qualification assessments will be performed.

The Treatment Phase will be comprised of two crossover periods separated by a 3 ± 1 day washout period. During each CRU admission and through the washout period, Subjects will continue a < 2-gram sodium diet and abstain from caffeine-containing products. At baseline (Day 0), subjects will be randomly assigned in a 1:1 ratio to 1 of 2 treatment sequences to receive intravenous (IV) furosemide or subcutaneous (SC) SCP-111 in the abdomen in Crossover Periods (i.e., IV followed by SC or vice versa). Blood samples will be collected to measure furosemide plasma concentrations at pre-dose and for 12 hours post-dose. Prior to dosing in each Treatment Phase, subjects will void their bladder completely and urine will be collected starting after the injection through 12 hours post injection to determine total urine output and urinary sodium and potassium excretion. Assessment of injection site pain will be conducted for IV and SC administration using an 11-point pain scale. Photographs of the injection site will be performed prior to the IV and SC injection (baseline) and 4 hours post IV administration and 6 hours post SC administration. In addition, a photograph will be taken of the IV and SC injection site for any adverse event (AE) (i.e., erythema, edema, pruritus, bruising) that occurs at the site at the time of the event. Subjects will remain domiciled in the CRU for each Treatment Phase through 12 hours after administration of study drug. After final assessments are performed, subjects may be discharged from the CRU if safety parameters are acceptable to the Investigator.

The Follow-up Phase will occur 24-48 hours (Day 5 ± 1) after discharge from the CRU following Crossover Period 2, completing Subjects' study participation.

1. TREATMENT

Study Drug:

- Intravenous Furosemide (IV Furosemide): Hospira, furosemide injection, solution 10 mg/mL (NDA 018667) (total dose = 80 mg) administered

intravenously 40 mg over 1 to 2 minutes by IV injection followed by a second dose of 40 mg over 1 to 2 minutes, 2 hours later (reference treatment).

- SCP-111 (furosemide injection), 80 mg/1 mL for subcutaneous administration: Total dose = 80 mg; administered as a subcutaneous injection (test treatment) via an autoinjector.

SCP-111, (furosemide injection), 80 mg/1 mL is a proprietary furosemide formulation that is isotonic and buffered to a neutral pH for subcutaneous administration.

Study Device: SCP-111 Autoinjector is an investigational single-entity drug-device combination product consisting of a prefilled syringe containing 1 mL of SCP-111 (furosemide injection), preloaded into a fixed single dose, disposable, two step mechanical autoinjector designed to administer the full dose to the subcutaneous tissue.

2. SAMPLE SIZE

In one study, the average coefficient of variation for furosemide AUC_{inf} following a 20 mg dose by the intravenous, oral, and sublingual routes of administration were 50%, 25%, and 33%, respectively (Haegeli, et al. 2007). In another study, the coefficient of variation for AUC_{inf} following 2-40 mg intravenous doses 2-hours apart was 31.6% and 30.6% for an 80 mg, 5-hour subcutaneous infusion (Sica DA, et al. 2018).

Assuming an intra-subject variability is approximately ½ of total variability for the primary pharmacokinetic parameter AUC_{inf} and the test reference would fall within 95% to 105% at a significance level of 0.05, a minimum sample size of 18 subjects would be required to establish bioequivalence between test and reference products (defined as 90% CI for AUC_{inf} Geometric mean ratio SC injection to IV injection of furosemide within 80-125% interval) with a priori statistical power of at least 90%.

21 subjects will be enrolled to ensure 18 subjects complete the study through the Follow-up Phase.

3. OBJECTIVES

3.1. Primary Objective:

To estimate the bioavailability of SCP-111 80 mg/1 mL administered as a subcutaneous injection via an autoinjector compared with an equivalent dose of furosemide administered as two 40 mg intravenous injections over one to two minutes, two hours apart.

3.2. Secondary Objectives:

- To describe the pharmacokinetics and pharmacodynamics of SCP-111 80 mg/1 mL administered as a subcutaneous injection via an autoinjector.
- To describe the safety and tolerability of SCP-111 80 mg/1 mL administered as a subcutaneous injection via an autoinjector.

4. DATA ANALYSIS CONSIDERATION

4.1. General Data Considerations:

The following conventions will be applied to analyses/data presentation:

All dates will be displayed in DDMMMYYYY format.

Repeat assessments will be considered unscheduled. Unscheduled visit data will be presented in Listings, but not in Tables and Figures.

For pharmacokinetic analyses, Phoenix® WinNonlin® version 8.2 or higher will be used. The statistical analyses will be performed using SAS release version 9.4 or higher.

All Tables, Listings and Figures (TLFs) will be produced in landscape format. In general, all data collected during the study will be presented in the data listings. Unless otherwise noted, the data will be sorted first by treatment, then by subject number, and then by date within each subject number. The number of variables presented in each listing can vary. Please refer to Mock tables, listings, and figures.

Data will be summarized by treatment where appropriate. The total number of subjects (N) in the treatment under the stated population will be displayed in the header of summary tables.

Data will be summarized using descriptive statistics for continuous variables. Unless otherwise stated, descriptive statistics will include number of subjects (n), mean, standard deviation (SD), minimum, median**,** and maximum. When n=0**,** except n, all other summary statistics will be kept blank. In case n=1, only n, minimum and maximum will be reported, and all other summary statistics will be kept blank. The statistic “Missing” will also be evaluated by enumerating the number of missing entries/subjects, if any at that visit, and presented as a summary statistic only for the resulting visits.

For categorical variables, data will be summarized using counts and percentages. The number [n] indicates the actual number of subjects with a particular value of a variable or event, which should always be less than or equal to the total number of subjects in the respective treatment [N]**.** The number and percentage of subjects with missing values for a variable/category/event will also be presented for the resulting visits under the “Missing” category. Percentage will be obtained by: % = (n/m) *100, where m is the number of subjects present at that visit. Unless otherwise stated, all percentages will be expressed to one decimal place. The number and

percentage of subjects will always be presented in the form XX (XX.X). Counts of zero in any category will be presented as “0” and if the percentage in any category to be presented is 100, it will be presented as “100”.

4.2. Pharmacokinetic Procedures

All the available data from the PK population will be used in the pharmacokinetic analyses. Pharmacokinetic calculations will be performed using non-compartmental methods.

If a subject’s pre-dose concentration is less than or equal to 5% of the C_{max} value for that subject in a period, then the subject’s data from that period without any adjustments will be included in all PK calculations. If the pre-dose value is greater than 5% of the C_{max} in a period, then the data from that period will be excluded, but the data will still be presented in the final study report.

Any sample concentration reported less than the assay limit of quantitation will be set to zero for use in the pharmacokinetic and statistical analyses. Pharmacokinetic and statistical analyses will be conducted on reported values. No concentration estimates are to be calculated for missing values.

Pharmacokinetic parameters (areas, times to peak, and elimination rates) will be calculated using the reported, rather than the scheduled, times of sample collection. Graphical presentations of individual subject results will also use the reported times of sample collection. Graphical presentations of mean results will use the scheduled times.

The following PK parameters will be estimated by non-compartmental methods from plasma samples:

Parameter:	**Definition:**
C_{max}	Maximum observed plasma concentration.
T_{max}	Time of maximum concentration (h), obtained directly from the observed concentration versus time data.
AUC_{last}	Area under the plasma concentration-time curve from time 0 to time of last measurable plasma concentration, calculated using the linear up/log down trapezoidal method.
AUC_{inf}	Area under the plasma concentration-time curve from 0-time extrapolated to infinity. $AUC_{inf} = AUC_{last} + C_{last}/ \lambda_z$; where C_{last} is the last measurable concentration, λ_z is elimination rate constant.
AUC_{ext}	The percentage of the AUC that is extrapolated beyond the last measurable concentration.

λ_z	Apparent plasma terminal-phase elimination rate constant. Estimated by linear regression of log concentration vs. time.
$t_{½}$	Terminal-phase half-life, calculated as ln(2)/ λ_z.
Vz/F	Apparent volume of distribution, terminal phase, for SCP-111, calculated as: $\frac{Dose}{\lambda_z * AUC_{inf}}$
V	Systemic volume of distribution, terminal phase, for IV furosemide, calculated as: $\frac{Dose}{\lambda_z * AUC_{inf}}$
CL/F	Apparent systemic clearance for SCP-111, calculated as: $\frac{Dose}{AUC_{inf}}$
CL	Systemic clearance for IV furosemide, calculated as: $\frac{Dose}{AUC_{inf}}$
F	Bioavailability ([AUC_{inf} SC SCP-111] / [AUC_{inf} IV furosemide])

A subject profile is to be excluded from AUC_{inf}, AUC_{ext}, λ_z, $t_{½}$, V, Vz/F, CL, CL/F, and F for cases that do not exhibit a terminal log-linear phase in the concentration versus time profiles or where the estimated $t_{½}$ is physiologically implausible.

4.3. Decimal Precision Convention:

The minimum and maximum statistics will be presented to the same number of decimal places as the original data. The mean and median, Confidence Intervals (CI) will be presented to one more decimal place than the original data, whereas the SD and Standard Error (SE) will be presented to two more decimal places than the original data. P-value will be presented with three decimal places. If p-value is closer to 0, then it should be reported as “<0.001” and if it is closer to 1, then it should be reported as “>0.999”.

4.4. HANDLING OF MISSING DATA

No plasma concentration estimates will be imputed for missing values. Missing values will be treated as if they were not scheduled for collection and only those

samples with reported concentration values will be included in the pharmacokinetic and statistical analysis.

Missing values for urine collections will not be imputed. Any subject with a missing value for any subinterval (e.g. 0-1 hour) involved in the 0-6, 0-8 or 0-12 results will be excluded from statistical analyses for the interval affected.

No imputation of missing data will be done for any other data. To handle missing or partial AE and concomitant medication dates, the following rules in 5.4.1 will be applied.

4.4.1. For Partial Start Dates:

1. If the year is unknown, then do not impute the date but assign a missing value.
2. If the month is unknown, then:
    i. If the year matches the year of the first dose date, then impute the month of the dose date.
    ii. Otherwise, assign “January.”
3. If the day is unknown, then:
    i. If the month and year match the month and year of the first dose date, then impute the day of the dose date.
    ii. Otherwise, assign “01.”

4.4.2. For Partial End Dates:

1. If the year is unknown, then do not impute the date but assign a missing value.
2. If the month is unknown, then assign “December.”
3. If the day is unknown, then assign the last day of the month.

After implementing the rules above, to determine whether AEs (or medications) with missing start or stop dates are pre-treatment or on/after first or second treatment, the following strategy will be used:

1. If both start date and stop date are missing, then the most conservative approach is taken, and the AE (or medication) is classified as treatment emergent (or concomitant medication).
2. If the start date is missing but the stop date is not missing and is on or after the day of study dose administration for Period 1 or 2, then the most conservative approach is taken, and the AE (or medication) is treatment emergent (or concomitant medication).
3. If the start date is missing but the stop date is not missing and is before the day of study dose for Period 1 and after the date of signed informed consent, then the AE (or medication) is not a TEAE (or prior medication).

4. If the start date is not missing but the stop date is missing, then the most conservative approach is taken, and medication is concomitant while the AE is defined by start date.

5. DEFINITIONS

Baseline Definition: Baseline for Period 1 is defined as Baseline Assessment (Day 0) (or Screening if missing) and for Period 2 as Pre-Period 2 Assessment (Day 3 ± 1).

Change from Baseline: The change from baseline values will be calculated as post-baseline value (p) minus the baseline value (b) for each Period 1 and 2.
Percent (%) Change from Baseline: The % change from baseline values will be calculated as (p - b)/b x 100 for each Period 1 and 2.

6. ENDPOINTS

6.1. Pharmacokinetic Endpoints

For IV administration of furosemide injection, USP (reference), PK blood samples will be collected as follows:

- First IV 1 to 2-minute injection dose: Pre-dose and 5, 15, 30, 45 minutes and 1, 1.5, 2 hours after **start** of first IV dose. The sample taken at 2 hours will be drawn immediately prior to the start of the second IV dose.
- Second IV 1 to 2-minute injection dose (new clock for PK sampling starts upon the start of administration of second IV dose): 5, 15, 30, 45 minutes and 1, 1.5, 2, 3, 4, 6, 8 and 10 hours after **start** of second IV dose.

For the SC administration of SCP-111 (test), PK blood samples will be collected as follows:

- Pre-dose and 5, 15, 30, 45 minutes and 1, 1.25, 1.5, 2, 3, 4, 5, 6, 8 and 12 hours after the SC injection

Individual pharmacokinetic parameters for furosemide will be summarized with descriptive statistics. Pharmacokinetic parameters including:

- the maximum observed plasma concentration (C_{max}),
- the area under the plasma concentration versus time curve from time 0 (pre-dose) to the last quantifiable time point (AUC_{last}),
- the AUC from time 0 (pre-dose) to time infinity (AUC_{inf}),
- the time to C_{max} (T_{max}), the terminal phase elimination rate constant (λz), the terminal elimination half-life (t½) for both SC SCP-111 (test) and IV furosemide (reference),

- the apparent systemic clearance (CL/F) for SC SCP-111 and systemic clearance (CL) for IV furosemide,
- the apparent systemic volume of distribution (Vz/F) for SC SCP-111

Systemic volume of distribution (V) for IV furosemide will be calculated using non-compartmental analysis.

Bioavailability of SC SCP-111 will be determined based on the geometric mean ratios of AUC_{last} and AUC_{inf} of IV furosemide (reference) and SC SCP-111 (test) and 90% confidence intervals on these ratios.

Geometric mean ratios of C_{max} of IV furosemide (reference) and SC SCP-111 (test) and 90% confidence intervals on these ratios will also be determined.

6.2. Pharmacodynamic Endpoints

Urine will be collected from spontaneous voids in 1-hour increments for the first 8 hours and in 2-hour increments up to 12 hours post-dose. Total urine output, urinary sodium, and potassium excretion for each collection interval ((0-1), (1-2), (2-3), (3-4), (4-5), (5-6), (6-7), (7-8), (8-10) and (10-12) hours after initiation of dosing) will be recorded.

The following PD parameters will be evaluated for IV furosemide and SC SCP-111 subcutaneous administration:

Urine Output:

- Measured at 0-6 hours, 0-8 hours, and 0-12 hours
- The sum of urine output for intervals 0-1, 1-2, 2-3, 3-4, 4-5 and 5-6 comprise 0–6-hour urine output.
- The sum of urine output for intervals 0-1, 1-2, 2-3, 3-4, 4-5, 5-6, 6-7 and 7-8 comprise 0–8-hour urine output.
- The sum of urine output for intervals 0-1, 1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8, 8-10 and 10-12 comprise 0–12-hour urine output.

Urinary Sodium Excretion:

- Measured at 0-6 hours, 0-8 hours, and 0-12 hours
- Calculated as: Urinary Sodium Excretion=Total Urine Output (L) × Sodium Concentration (mEq/L)

Urinary Potassium Excretion:

- Measured at 0-6 hours, 0-8 hours, and 0-12 hours
- Calculated as: Urinary Potassium Excretion=Total Urine Output (L) × Potassium Concentration (mEq/L)

Example Calculation:

- **Interval: 0-1 hour**

  Total Urine Output=0.5 L

  Sodium Concentration=100 mEq/L

  Urinary Sodium Excretion=0.5 L×100 mEq/L=50 mEq

- **Interval: 1-2 hours**

  Total Urine Output=0.4 L

  Sodium Concentration=90 mEq/L

  Urinary Sodium Excretion=0.4 L×90 mEq/L=36 mEq

Continue this calculation for each subsequent interval (e.g., 0-1, 1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8, 8-10, and 10-12 hours) to obtain the total urinary sodium excretion for each time period.

Same calculations are conducted to calculate urinary potassium excretion for each interval.

The sum of intervals 0-1, 1-2, 2-3, 3-4, 4-5 and 5-6 comprise 0-6 hour urinary sodium/potassium excretion.

The sum of intervals 0-1, 1-2, 2-3, 3-4, 4-5, 5-6, 6-7 and 7-8 comprise 0-8 hour urinary sodium/potassium excretion.

The sum of intervals 0-1, 1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8, 8-10 and 10-12 comprise 0-12 hour urinary sodium/potassium excretion.

6.3. Safety and tolerability

Adverse Events (AEs) and Serious Adverse Events (SAEs) will be grouped by body system and summarized. The incidence (number and percentage of subjects) of adverse events and serious adverse events will be presented overall and by MedDRA System Organ Class (SOC) and Preferred Term (PT).

6.4. Injection Site Pain

Injection site pain will be assessed using an 11-point scale where 0 is equivalent to no pain and 10 is equivalent to the worst possible pain. For IV administration, the 11-point pain scale will be performed pre-dose, after IV needle placement, immediately after 1st dose, and 15 and 30 minutes after 1st dose, immediately after 2nd dose, and 15 and 30 minutes and 4 and 10 hours after the 2nd dose. For SC administration, the 11-point pain scale will be performed pre-dose, immediately after dose and 15 and 30 minutes and 6 and 12 hours after dose. Pain requiring treatment, withdrawal from the study or discontinuation of study drug will also be recorded as an adverse event (AE).

7. INTERIM ANALYSIS

There will be no formal interim analysis.

8. ANALYSIS POPULATIONS

The statistical analyses and summaries will be based on the study populations detailed below.

The subject demographics, baseline characteristics and the other endpoints will be based on the Full Analysis Set (FAS) population. The PK Analysis will be performed on the Bioavailability (BA) population.

- **Full Analysis Set (FAS) Population**: This includes all participants who were randomized in the study, regardless of whether they completed the study as per protocol.
- **Pharmacokinetic (PK) Population** will include all randomized subjects who receive full 80 mg dose of furosemide study drug AND have sufficient samples collected for estimation of pharmacokinetic parameters.
- **Bioavailability (BA) Population** will include all subjects in the PK population who have a sufficient number of samples collected for estimation of pharmacokinetic parameters after receiving BOTH IV furosemide and SC SCP-111.

All Safety analyses will be based on the safety population.

- **Safety Population** will include all randomized subjects who received at least 1 dose of either study drug and have provided at least 1 post-baseline safety assessment.

9. STATISTICAL ANALYSIS

This is an open-label, single-dose, randomized, two-way (two-period) crossover Study to Compare the Pharmacokinetics and Pharmacodynamics of SCP-111 (furosemide injection). The SAP takes precedence over the protocol.

9.1. Pharmacokinetic Analyses

The analyses will be performed on subjects in the Bioavailability Population.

Derived plasma PK parameters will be tabulated and summarized by treatment. Descriptive statistics for PK parameters (C_{max}, T_{max}, AUC_{last}, AUC_{inf}, AUC_{ext}, λ_z, $t_{½}$, V, Vz/F, CL, CL/F, and F) will include the arithmetic mean, CV%, SD of the arithmetic mean, median, minimum, maximum, and N. The geometric mean and geometric CV% will be estimated for C_{max}, AUC_{last}, and AUC_{inf}, only.

Statistical analyses will be performed using the General Linear Models (GLM) procedure of the SAS statistical program (PC version 9.4). The naturally log-transformed (ln-transformed) pharmacokinetic parameter estimates (for C_{max}, AUC_{last}, and AUC_{inf}, only), as well as the concentrations at each scheduled sample time will be evaluated by analysis of variance.

The statistical model will contain the main effects of sequence, subject nested within sequence, treatment and period. F-ratios for testing main effects will be constructed using the mean square term for the effect as the numerator and the mean square error term from the ANOVA as the denominator. The F-ratio to test for sequence effects will be constructed using the type III mean square term for sequence as the numerator and type III mean square for subjects nested within sequence as the denominator. Hypothesis testing will be conducted at $\alpha = 0.05$, except in the case of sequence effects which uses $\alpha = 0.10$.

The PK Analysis report will discuss any statistically significant effect found for AUC or C_{max}.

The intra-subject coefficient of variation will be estimated from the mean square error term (MSE) of the ln-transformed (loge) results as:

$$100\% * SQRT(e^{MSE} - 1)$$

Confidence Intervals (90%) for the area under the curve (AUC) and peak concentration (C_{max}) comparisons are to be calculated by the t-test approach (2,1 sided) at $\alpha = 0.10$ overall, $\alpha = 0.05$ each side:

$$\text{Interval Lower Limit} = (X_{SC} - X_{IV}) - Se * t_{\alpha/2}$$

$$\text{Interval Upper Limit} = (X_{SC} - X_{IV}) + Se * t_{\alpha/2}$$

Where X_{SC}, X_{IV} are the SC SCP-111 and IV furosemide treatment least-squares means, respectively.

SE is the standard error of the estimated difference between means from the SAS estimate statement.

$t_{\alpha/2}$ is the critical value from the t-distribution with degrees of freedom that of the error term and $\alpha = 0.10$.

For ln-transformed data, the interval will be calculated from the ANOVA results on the transformed values and then exponentiated to convert to the non-transformed scale:

Interval Limit = e(ln-transformed interval limit)

The intervals will be computed for the "true" mean treatment differences and true geometric mean ratios (from logarithmic transformation).

Similarly, the exponentiated difference between the SC SCP-111 and IV furosemide least squares means from ln-transformed results provided an estimate of the geometric mean test-to-reference ratio.

If there are multiple dosing groups, and subjects within each group receive their first and second doses on the same dates, adjustments to the statistical model for PK parameter analyses will be made.

9.1.1. Criteria for Assessment of Relative Bioavailability

90% confidence intervals for AUC_{last} and AUC_{inf} will be calculated, with intervals between 80% and 125% indicating equivalent relative bioavailability.

9.2. Pharmacodynamic Analyses

Total urine output, total urinary sodium excretion, and total urinary potassium excretion from times 0-6, 0-8 and 0-12 hours will be tabulated by summing the concentration*volume of each collection interval across the time span specified and summarized using descriptive statistics including N, mean, standard deviation (SD), coefficient of variation (CV%), median, minimum, maximum, geometric mean and geometric CV% analyzed by treatment in the BA population.

A repeated measures, mixed model analysis will be used to evaluate the mean difference between SC and IV across all time points. This analysis will account for within-subject correlations and between-subject variability. The model will include fixed effects for treatment (SC vs IV), time, and treatment-by-time interaction, and a random effect for subjects.

- 95% Confidence Intervals (CI) for the difference between SC and IV administration will be calculated for all measured parameters.
- P-values will be determined for the comparison of subcutaneous and intravenous administration methods, using the mixed model analysis.
- The means and 95% CIs of the parameters will be plotted at the specified time points: 0-6,0-8 and 0-12 hr.

9.3. Analysis of Safety

All safety analyses will be performed on the Safety Analysis Set. All safety data will be listed in individual subject listings by treatment and subject.

For the evaluation of safety parameters, the continuous variables will be summarized descriptively per treatment, period, time point, and overall, by N, arithmetic mean, median, SD, and minimum and maximum values. Categorical variables will be presented in frequency tables with the counts of observations and corresponding percentages.

9.3.1. Adverse Events

Adverse events will be mapped to the Medical Dictionary for Regulatory Activities (MedDRA™) dictionary.

AEs will be assigned to each treatment period as follows:

- **Treatment-emergent AEs in Period 1**
  - All AEs with start date/time on or after the start of dosing in Period 1 and before the start of the washout period (if not missing) having been absent in pre-treatment (or) worsen in severity relative to the pre-treatment state following administration of study drug in Period 1.
  - AEs that emerge during the washout period and/or are causally related to the study treatment administered in the time preceding the washout period.

- **Treatment-emergent AEs in Period 2**
  - All AEs with start date/time on or after the start of dosing in Period 2 having been absent in washout period (or) worsen in severity relative to the pre-treatment state following administration of study drug in Period 2.
  - AEs that emerge during the Follow-up Phase and/or are causally related to the study treatment administered in the time preceding the Follow-up Phase.

AE summaries will be displayed by treatment for:

- **Overall AE Summary**
  - A general summary that includes all TEAEs reported throughout the study, regardless of the treatment period.
- **Period-Specific AE Summaries**
  - Separate TEAE summaries for each treatment period (1 and 2).

The AEs that occur prior to treatment in Period 1 will be summarized in listings and not included in TEAE summary tables. This helps in understanding their impact without confounding the safety profile of the specific treatment periods. All other adverse events will be considered ‘pre-treatment’.

In the case where it is not possible to define an AE as being treatment emergent or not, the AE will be classified as treatment emergent as the most conservative approach.

Analyses of AEs will be performed for those events that are considered treatment emergent. The AE tables will include the number and percentage of subjects with at least one AE, the number of AEs by MedDRA primary SOC (sorted in decreasing order of the total frequency) and MedDRA PT (sorted in descending order of the total frequency within each SOC) unless otherwise indicated. A subject with more than one occurrence of the same AE in a particular SOC/PT will be counted only once in the total of those experiencing AEs in that particular SOC /PT.

The AE Relationship to Study Treatment will be combined where Related includes the categories Possibly Related, Probably Related, Definitely Related and Unrelated includes Not Related.

For the overall summary tables and the AE tables summarized by intensity, a subject who has more than one AE within a particular category (SOC or SOC /PT combination) with differing intensities will be counted for each recorded intensity.

An overview table of AEs will be presented by treatment with the number and percentage of subjects with at least one:

- Treatment-emergent AE (TEAE)
- Treatment-related TEAE
- TEAE by severity: mild, moderate, severe
- Serious TEAE
- TEAE leading to study withdrawal
- TEAE leading to death

9.3.2. Serious Adverse Events

Treatment Emergent SAEs will be listed, sorted by treatment period, subject identification, and onset date. In addition, the incidence (number and percentage of subjects) with SAEs by MedDRA SOC and PT will be displayed overall and by treatment for each period.

9.3.3. Injection Site Pain

Injection site pain assessed pre-dose and post-dose for both Intravenous (IV) and Subcutaneous (SC) administration will be summarized using descriptive statistics. Descriptive statistics, including mean, median, standard deviation, and minimum maximum, will be calculated for each time point for both IV and SC administrations. These statistics will summarize the pain score distribution, central tendency, and variability.

9.3.4. Other Safety Analyses

9.3.4.1. Clinical Laboratory Evaluation

This section outlines the designated laboratory evaluations to be conducted at predetermined visits during the study and describes how these results will be systematically summarized.

Included Laboratory Tests:

- **Electrolytes and Metabolites**:
  - Sodium (Na+)
  - Potassium (K+)
  - Calcium (Ca2+)
  - Chloride (Cl-)
  - Magnesium (Mg2+)
  - Carbon Dioxide (CO2)
  - Blood Urea Nitrogen (BUN)
  - Serum Creatinine
  - Glucose
- **Hematological Parameters**:
  - Hemoglobin
  - Hematocrit

eGFR will be calculated using the simplified Modification of Diet in Renal Disease (sMDRD) equation and included in the clinical laboratory summaries and analyses.

eGFR (mL/min/1.73 m²) = 175 × (Serum Creatinine (mg/dL))$^{-1.154}$ × (Age)$^{-0.203}$ × (0.742 if female) × (1.212 if African American)

Where:

- Serum Creatinine is measured in mg/dL
- Age is in years

All parameters will be summarized using descriptive statistics. Summaries will be provided by the treatment for pre-dose and post-dose in each treatment period. Data will be presented as both raw values and as changes from baseline.

Period-Specific Analysis:

Changes during each treatment period will be evaluated. For Period 1, this involves comparing baseline (Screening or Baseline Assessment (Day 0), if baseline data is missing) with Treatment Crossover Period 1 (Day 0). For Period 2, changes from baseline (Pre-Period 2 Assessment (Day 3 ± 1) to Treatment Crossover Period 2 (Day 3 ± 1) will be assessed.

Frequency tables will classify values as low, normal, or high based on reference ranges. These tables will provide insights into the distribution of values across these classifications.

All abnormal laboratory values will be listed to highlight noteworthy deviations.

Timetable for Laboratory Assessments:

- **Baseline Assessment (Day 0)**: Initial laboratory assessments prior to any treatment.
- **Treatment Crossover Period 1 (Day 0)**: Laboratory assessments 12 hours after the study drug is administered in the first treatment phase.
- **Pre-Period 2 Assessment (Day 3 ± 1)**: Laboratory assessments conducted before the commencement of the second treatment phase.
- **Treatment Crossover Period 2 (Day 3 ± 1)**: Laboratory assessments 12 hours after the study drug is administered in the second treatment phase.

9.3.4.2. Vital Signs

Systolic and diastolic blood pressures (mmHg), heart rate (beats/min), and body temperature (°C) will be summarized with descriptive statistics at each timepoint and changes from baseline including mean, standard deviation, median, minimum, and maximum values.

Data will be analyzed by timepoint for each treatment group (IV and SC). A p-value for between-treatment group comparison will be provided at each timepoint to assess statistical significance of any observed differences.

All data will be listed by treatment, treatment sequence, subject, visit, and time point.

9.3.4.3. 12-lead ECG

ECG parameters will be summarized with descriptive statistics for the overall population at baseline. Data for each subject will be listed.

9.3.4.4. Physical Examinations

Physical examination will be presented for the overall group collected at screening and follow-up phase, by treatment across both periods highlighting shifts from normal to abnormal (clinically significant or not) at pre-dose and post-dose timepoints and listing by treatment sequence.

9.3.5. Other Analyses

9.3.5.1. Subject Disposition and Study Discontinuations Overview

This section of the study will comprehensively account for all participants from the point of providing informed consent through to the completion of the study or any instances of discontinuation. Detailed summaries and listings will be organized to reflect the progress and attrition of subjects throughout the study, with an emphasis on clarity and thoroughness in reporting. These summaries will be stratified by treatment sequence and presented collectively for each treatment.

Summaries will include:

- The total count of subjects who screened for the study, defined as those who provided informed consent.
- The number of subjects who were randomized into the study.
- The count of subjects who received treatment in each respective period.
- The tally of subjects who successfully completed each treatment period.
- The overall number of subjects who completed the entire study.
- Detailed breakdown of subjects who discontinued the study treatment, categorized by the primary reason for discontinuation and when.
- Enumeration of subjects who withdrew from the study entirely, categorized by their main reason for doing so and when.

Analysis Populations. The number and percentage of subjects in each of the analysis populations will be presented for all randomized subjects.

Individual listings will be prepared, organized by treatment, treatment sequence, and subject identification. These listings will provide details regarding subject progression and withdrawal reasons.

For subjects who discontinue, detailed listings will be provided, specifying each individual's reason for withdrawal.

Moreover, a separate listing will be created to document screening failures, along with the reasons for their non-inclusion in the treatment phase of the study.

9.3.5.2. Analysis of Demographics and Baseline Characteristics

Demographic characteristics will be summarized overall and by treatment sequence for each treatment for the following: sex, race, ethnicity, age, weight, height, BMI.

Specifications for computation for Age [years]:

- (date of given informed consent – date of birth + 1) / 365.25

Medical History. Medical history will be coded using Medical Dictionary for Regulatory Activities (MedDRA) version 27.0 and will be displayed in terms of frequency tables overall and by treatment sequence: ordered by primary SOC and PT in alphabetical order.

When Chronic Kidney disease is reported, the stage will be tabulated and when Heart Failure is reported, NYHA Class will be tabulated.

9.3.5.3. Prior and Concomitant Medications

This section details the approach for recording and analyzing all medications before and during the study. All medications will be coded using WHODrug GLOBALB3Mar23.

Prior Medications: Defined as medications taken before the screening visit and ended on the day before the first trial drug dose. All medications taken within 7 days of screening will be noted.

Concomitant Medications: Includes non-trial medications taken at any time during the trial, either continuing from before or starting after the first trial drug dose. Summaries and listings of all prior and concurrent medications will be provided for all subjects, detailed by treatment sequence, PT, and ATC 4th level.
If a medication starts before study entry and continues through the study that will be considered both as prior and concomitant.
Prior and concomitant medications will be summarized by treatment sequence and overall. All medications will be listed by treatment sequence and subject.

9.3.5.4. Protocol Deviations

Protocol deviations will be listed by subject, treatment, date of protocol deviation, protocol deviation category, protocol deviation description, investigator assessment, sponsor assessment and action taken.

10. REFERENCES

Haegeli, Laurent; Brunner-La Rocca, Hans Peter; Wenk, Markus; Pfisterer, Matthias; Drewe, Jurgen; Krahenbuhl, Stephan (2007): Sublingual administration of furosemide: new application of an old drug. In *British journal of clinical pharmacology* 64 (6), pp. 804–809. DOI: 10.1111/j.1365-2125.2007.03035.x.

Sica, D. A., Muntendam, P., Myers, R. L., Ter Maaten, J. M., Sale, M. E., de Boer, R. A., & Pitt, B. (2018). Subcutaneous Furosemide in Heart Failure: Pharmacokinetic Characteristics of a Newly Buffered Solution. *JACC Basic Transl Sci, 3*(1), 25-34. doi:10.1016/j.jacbts.2017.10.001