Protocol B9531002

A single-dose, open-label, randomized, 2-way, cross-over pivotal bioequivalence study to qualify manufacturing site transfer from Viatris to Neolpharma, for Spironolactone/Hydrochlorothiazide film coated tablets in healthy adult participants under fasted conditions

[redacted]

Statistical Analysis Plan
(SAP)

Version: 1

Date: 17 Nov 2023

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NOTE: *Italicized* text within this document has been taken verbatim from the Protocol.

1. VERSION HISTORY

Table 1. Summary of Changes

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
1 / 17 Nov 2023	Original 28 Sep 2023	N/A	N/A

2. INTRODUCTION

Spironolactone/Hydrochlorothiazide film coated tablets are a combination of 2 diuretic agents spironolactone and hydrochlorothiazide indicated for treatment of essential hypertension, congestive heart failure, liver cirrhosis, nephrotic syndrome and other edematous conditions. Spironolactone and hydrochlorothiazide have different but complementary mechanisms and sites of action, thereby providing additive diuretic and antihypertensive effects.

The purpose of this study is to assess the bioequivalence (BE) between Spironolactone/Hydrochlorothiazide film coated tablets manufactured at Viatris and Spironolactone/Hydrochlorothiazide film coated tablets manufactured at Neolpharma.

This SAP provides the detailed methodology for summary and statistical analyses of the data collected in Study B9531002.

2.1. Modifications to the Analysis Plan Described in the Protocol

None.

2.2. Study Objectives, Endpoints, and Estimands

The following are the objectives and endpoints in this study. Estimand framework will not be applied to this Phase 1 study in healthy participants.

Objectives	*Endpoints*
Primary:	***Primary:***
• *To demonstrate bioequivalence between Spironolactone/Hydrochlorothiazide 25 mg/25 mg film coated tablets manufactured at the proposed site (Neolpharma) vs Spironolactone/Hydrochlorothiazide 25 mg/25 mg film coated tablets manufactured at current site (Viatris) under fasting conditions in healthy adult participants.*	• *C_{max}, AUC_{inf} (or AUC_{last} if AUC_{inf} cannot be reliably estimated) of Spironolactone and Hydrochlorothiazide.*
Secondary:	***Secondary:***
• *To further characterize PK of Spironolactone and Hydrochlorothiazide.* • *To evaluate the safety and tolerability of Spironolactone/Hydrochlorothiazide film coated tablets.*	• *$t_{½}$ (if data permit) and T_{max} of Spironolactone and Hydrochlorothiazide.* • *AEs, clinical laboratory tests, vital signs, and ECGs.*

2.3. Study Design

This will be an open-label, randomized, single-dose, 2-treatment, 2-period, 2-sequence, crossover study in adult healthy male and/or female participants. Approximately 40 participants will be enrolled in the study (20 in each treatment sequence). Dropouts for non-safety reasons or non-evaluable participants may be replaced at the discretion of the sponsor and investigator. Screening evaluation will occur within 28 days prior to the first dose of study medication. Participants will be assigned to 1 of the following 2 sequences according to a computer-generated randomization schedule.

Table 2. Treatment Sequence

Sequence	*Number of Participants*	*Period 1*	*Washout Period*	*Period 2*
1	*20*	*Treatment A*	*At least 4 days*	*Treatment B*
2	*20*	*Treatment B*		*Treatment A*

Treatment A: Spironolactone/Hydrochlorothiazide 25 mg/25 mg film coated tablets manufactured at the current site (Viatris) under fasting conditions.

Treatment B: Spironolactone/Hydrochlorothiazide 25 mg/25 mg film coated tablets manufactured at the proposed site (Neolpharma) under fasting conditions.

3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

3.1. Primary Endpoints

The primary endpoints of the study are plasma C_{max}, AUC_{inf} (or AUC_{last} if AUC_{inf} cannot be reliably estimated) of Spironolactone and Hydrochlorothiazide. Adjusted geometric mean ratios of C_{max}, AUC_{inf} and AUC_{last} will be derived.

PK parameters will be derived from the concentration-time profiles as shown in Table 3. Actual PK sampling times will be used in the derivation of PK parameters. In the case that

actual PK sampling times are not available, nominal PK sampling time will be used in the derivation of PK parameters.

Table 3. Plasma PK Parameters Definitions

Parameter	***Definition***	***Method of Determination***
AUC_{last}	*Area under the plasma concentration-time profile from time 0 to time of the last quantifiable concentration (C_{last})*	*Linear-log trapezoidal rule*
AUC_{inf}[a]	*Area under the plasma concentration-time profile from time 0 extrapolated to infinite time*	$AUC_{last} + (C_{last}*/k_{el})$, *where $C_{last}*$ is the predicted plasma concentration at the last quantifiable time point estimated from the log-linear regression analysis.*
C_{max}	*Maximum plasma concentration*	*Observed directly from data*
T_{max}	*Time for C_{max}*	*Observed directly from data as time of first occurrence*
$t_{½}$[a]	*Terminal elimination half-life*	$Ln(2)/k_{el}$, *where k_{el} is the terminal phase rate constant calculated by a linear regression of the log-linear concentration-time curve. Only those data points judged to describe the terminal log-linear decline will be used in the regression*

a. If data permits.

3.2. Secondary Endpoints

3.2.1. Other PK parameters

Other PK parameters of Spironolactone and Hydrochlorothiazide, including $t_{1/2}$ (if data permit) and T_{max} will be derived.

3.2.2. Safety endpoints

The following data will be considered in standard safety summaries (see protocol for collection days, baseline assessment, and list of parameters):

- Adverse events (AE)
- Laboratory data
- Vital signs data
- ECG results

3.2.2.1. Adverse Events

Any adverse events occurring following start of treatment will be considered as treatment emergent adverse event (TEAE). Events that occur during follow-up within the lag time of up to 35 days after the study drug dose will be counted as treatment emergent and attributed to the last treatment taken. Events that occur during the washout period between study periods will be counted as treatment emergent and attributed to the previous treatment taken. The

time period for collecting AEs ("active collection period") for each participant begins from the time the participant provides informed consent.

3.2.2.2. Laboratory Data

Safety laboratory tests will be performed as described in the protocol.

The baseline measurement is the last predose measurement prior to administration of study drug in Period 1.

3.2.2.3. Vital Signs

Supine blood pressure (BP) and pulse rate (PR) will be measured at times specified in the SoA given in the protocol.

The baseline measurement is the last predose measurement prior to administration of study drug in Period 1.

3.2.2.4. Electrocardiograms

QT interval, QTcF, PR, QRS and heart rate (HR) will be recorded at each assessment time indicated in the SoA given in the protocol. If not supplied, QTcF will be derived using Fridericia's heart rate correction formula:

QTcF = QT / (RR)^(1/3) where RR = 60/HR (if not provided)

The baseline value is the last predose measurement on prior to administration of study drug in Period 1.

3.3. Other Safety Endpoint(s)

None.

3.4. Exploratory Endpoints

None.

3.5. Baseline Variables

Baseline characteristics will be collected according to the SoA as specified in the protocol.

4. ANALYSIS SETS (POPULATIONS FOR ANALYSIS)

Data for all participants will be assessed to determine if participants meet the criteria for inclusion in each analysis population prior to releasing the database and classifications will be documented per standard operating procedures.

For purposes of analysis, the following analysis sets are defined:

Participant Analysis Set	***Description***
Enrolled	*"Enrolled" means a participant's, or their legally authorized representative's, agreement to participate in a clinical study following completion of the informed consent process and randomization.*

Participant Analysis Set	***Description***
Full analysis set	*All participants randomly assigned to study intervention and who take at least 1 dose of study intervention.*
Safety analysis set	*All participants randomly assigned to study intervention and who take at least 1 dose of study intervention. Participants will be analyzed according to the product they actually received.*
PK Concentration Set	All participants who take at least 1 dose of study intervention and in whom at least 1 concentration value is reported.
PK Parameter Set	All participants who take at least 1 dose of study intervention and in whom at least 1 of the PK parameters of primary interest are reported.

5. GENERAL METHODOLOGY AND CONVENTIONS

Final analysis will be performed after study participant data set release following last participant last visit.

5.1. Hypotheses and Decision Rules

The alternative hypothesis of BE (H_1: $\theta_L <= \mu_T - \mu_R <= \theta_U$), and the null hypothesis of inequivalence (H_o: $\mu_T - \mu_R < \theta_L$ or $\mu_T - \mu_R > \theta_U$) can be expressed as the following 2 separate 1-sided hypotheses:

H_{oA}: $\mu_T - \mu_R < \theta_L$
H_{1A}: $\theta_L <= \mu_T - \mu_R$

H_{oB}: $\mu_T - \mu_R > \theta_U$
H_{1B}: $\mu_T - \mu_R <= \theta_U$

Where μ_T and μ_R represent the average BA on a log scale for the Test and Reference products respectively and [θ_L, θ_U] defines the BE range.

BE of the Test treatment to Reference treatment will be concluded if the 90% CIs for the ratios of adjusted geometric means for both spironolactone and hydrochlorothiazide AUC_{inf} and C_{max} fall entirely within the acceptance region of (80%, 125%).

5.2. General Methods

5.2.1. Analyses for Binary/Categorical Endpoints

For binary or categorical variables, number of participants, numbers and percentages of participants meeting the categorical criteria will be presented in accordance with the Clinical Data Interchange Standards Consortium and Pfizer Standards (CaPS).

5.2.2. Analyses for Continuous Endpoints

For continuous variables, the data will be summarized using the number of participants, mean, median, standard deviation (SD), minimum, and maximum in accordance with the CaPS. For appropriate PK parameters, geometric mean and geometric coefficient of variation (%CV) will also be summarized.

5.3. Methods to Manage Missing Data

5.3.1. Pharmacokinetic Data

Methods to handle missing PK data are described below.

Concentrations Below the Limit of Quantification:

In all data presentations (except listings), concentrations below the limit of quantification (BLQ) will be set to zero. (In listings BLQ values will be reported as "<LLQ", where LLQ will be replaced with the value for the lower limit of quantification.).

Deviations, Missing Concentrations and Anomalous Values:

In summary tables and plots of median profiles, statistics will be calculated having set concentrations to missing if one of the following cases is true:

1. A concentration has been collected as ND (ie, not done) or NS (ie, no sample).
2. A deviation in sampling time is of sufficient concern or a concentration has been flagged as anomalous by the pharmacokineticist.

Note that summary statistics will not be presented at a particular time point if more than 50% of the data are missing.

An anomalous concentration value is one that, after verification of bioanalytical validity, is grossly inconsistent with other concentration data from the same individual or from other participants. For example, a BLQ concentration that is between quantifiable values from the same dose is considered as anomalous. Anomalous concentration values may be excluded from PK analysis at the discretion of the PK analyst or pharmacokineticist.

PK Parameters:

Actual PK sampling times will be used in the derivation of PK parameters. If a PK parameter cannot be derived from a participant's concentration data, the parameter will be coded as NC (ie, not calculated). (Note that NC values will not be generated beyond the day that a participant discontinues). In summary tables, statistics will be calculated by setting NC values to missing; and statistics will be presented for a particular treatment with ≥3 evaluable measurements. PK parameter analyses will not be performed for a particular parameter if more than 50% of the data are NC.

If an individual participant has a known biased estimate of a PK parameter (due for example to an unexpected event such as vomiting before all the compound is adequately absorbed from the gastrointestinal tract), this will be footnoted in summary tables and will not be included in the calculation of summary statistics or statistical analyses.

5.3.2. Safety Data

For the analysis of safety endpoints, the standard rules for imputation according to CaPS will be applied.

6. ANALYSES AND SUMMARIES

6.1. Primary Endpoints

The primary endpoints C_{max} and AUC_{inf} (if data permits, otherwise AUC_{last}) will be summarized descriptively by treatment and will include the set of summary statistics as specified in Table 4. Box and whisker plots for individual participant parameters (C_{max}, AUC_{inf} and AUC_{last}) will be plotted by treatment and overlaid with geometric means.

Natural log transformed PK parameters (C_{max}, AUC_{inf} (or AUC_{last} if AUC_{inf} cannot be reliably estimated)) will be analyzed using a mixed effect model with sequence, period and treatment as fixed effects and participant within sequence as a random effect. Estimates of the adjusted mean differences (Test-Reference) and the corresponding 90% CIs will be obtained from the model. The adjusted mean differences and the 90% CIs for the differences will be exponentiated to provide estimates of the ratio of adjusted geometric means (Test/Reference) and the 90% CIs for the ratios.

For bioequivalence assessment of Spironolactone/Hydrochlorothiazide 25/25 mg film coated tablets between proposed site (Neolpharma) and current site (Viatris), bioequivalence of the Test treatment (Treatment B) relative to Reference treatment (Treatment A) will be concluded if the 90% confidence intervals for the ratio of adjusted geometric means of Test treatment (Treatment B) relative to Reference treatment (Treatment A) for C_{max}, AUC_{inf} (or AUC_{last} if AUC_{inf} cannot be reliably estimated) fall wholly within (80%, 125%).

PK parameters will be summarized descriptively in accordance with Pfizer data standards for the PK Parameter Set, as data permit. Missing values will be handled as detailed in Section 5.3.1. Each PK parameter will be summarized and will include the set of summary statistics as specified in Table 4.

Table 4. PK Parameters to be Summarized Descriptively

Parameter	Summary Statistics
AUC_{inf}, AUC_{last}, C_{max}	N, arithmetic mean, median, SD, %CV, minimum, maximum, geometric mean and geometric %CV
T_{max}	N, median, minimum, maximum
$t_{½}$	N, arithmetic mean, median, SD, %CV, minimum, maximum

Supporting data from the estimation of $t_{½}$ and AUC_{inf} will be listed by analyte and group: terminal phase rate constant (k_{el}); goodness of fit statistic from the log-linear regression (r^2); the percent of AUC_{inf} based on extrapolation (AUC_{extrap}%); and the first, last, and number of time points used in the estimation of k_{el}. This data may be included in the clinical study report.

Presentations for plasma concentrations using the PK Concentration Set will include:

- A listing of all concentrations sorted by participant ID, treatment and nominal time postdose. The concentration listing will also include the actual times. Deviations from the nominal time will be provided in a separate listing.

- A summary of concentrations by treatment and nominal time postdose, where the set of statistics will include n, mean, median, SD, %CV, minimum, maximum and the number of concentrations above the LLQ.

- Median concentrations time plots (on both linear and semi-log scales) against nominal time postdose by treatment (all treatments on the same plot per scale, based on the summary of concentrations by treatment and time postdose).

- Mean concentrations time plots (on both linear and semi-log scales) against nominal time postdose by treatment (all treatments on the same plot per scale, based on the summary of concentrations by treatment and time postdose).

- Individual concentration time plots by treatment (on both linear and semi-log scales) against actual time postdose (there will be separate spaghetti plots for each treatment per scale).

- Individual concentration time plots by participant (on both linear and semi-log scales) against actual time postdose [there will be separate plots for each participant (containing all treatments) per scale].

6.2. Secondary Endpoints

6.2.1. Other PK parameters

Other PK parameters including T_{max} and $t_{1/2}$ will be summarized descriptively by treatment and will include the set of summary statistics as specified in Table 4.

6.2.2. Safety endpoints

All safety analyses will be performed on the Safety Analysis Set.

Safety data will be presented in tabular and/or graphical format and summarized descriptively by treatment, where appropriate.

6.2.2.1. Adverse Events

Adverse events will be reported in accordance with the CaPS and listed by treatment, where appropriate.

Participant discontinuations due to adverse events will be detailed by treatment. Data will be reported in accordance with the CaPS.

6.2.2.2. Laboratory Data

Laboratory data will be listed by treatment in accordance with the CaPS.

6.2.2.3. Vital Signs

Vital signs data will be databased and available upon request.

6.2.2.4. Electrocardiograms

ECG data will be databased and available upon request.

6.3. Other Safety Summaries and Analyses Endpoint(s)

None.

6.4. Exploratory Endpoints

None.

6.5. Subset Analyses

There are no planned subset analyses.

6.6. Baseline and Other Summaries and Analyses

6.6.1. Demographic Summaries

Demographic characteristics will be summarized for safety population in accordance with the CaPS.

6.6.2. Study Conduct and Participant Disposition

Participants evaluation groups will show end of study participant disposition. Frequency counts will be supplied for participant discontinuation(s) by treatment. Data will be reported in accordance with the CaPS.

6.6.3. Study Treatment Exposure

Study treatment exposure will be listed.

6.6.4. Concomitant Medications and Nondrug Treatments

All concomitant medication(s) as well as non-drug treatment(s) will be reported in the listings.

7. INTERIM ANALYSES

No interim analysis will be conducted for this study. As this is an open-label study, the sponsor may conduct reviews of the data during the course of the study for the purpose of safety assessment, facilitating PK modeling, and/or supporting clinical development.

APPENDICES

Appendix 1. Summary of Analyses

Endpoint	Population	Data Inclusion and Rules for Handling Intercurrent Events and Missing Data	Analysis Method
Geometric mean ratio of C_{max}, AUC_{inf}, AUC_{last}	PK Parameter Analysis Set	Observed and imputed (Section 5.3.1) data	Mixed effect model
PK parameters	PK Parameter Analysis Set	Observed and imputed (Section 5.3.1) data	Descriptive statistics
PK concentrations	PK Concentration Analysis Set	Observed and imputed (Section 5.3.1) data	Descriptive statistics
Safety endpoints	Safety Analysis Set	Observed and imputed (Section 5.3.2) data	Descriptive statistics

Appendix 2. SAS Code for Analyses

An example of PROC MIXED code is provided below.

For bioequivalence:

```
proc mixed data=tab.pk;
        class seq period trt participant;
        model log&var=sequence period trt / ddfm=KR;
        random participant(seq) / subject=participant(seq);
        lsmeans trt;
        estimate 'B vs A' trt -1 1 /cl alpha=0.1;

        ods 'Estimates' out=est&var;
        ods 'lsmeans' out=ls&var;
        ods 'covparms' out=cov&var;
        ods 'tests3' out=tst&var;
run;

/* Letter assignments for treatments (trt) within the estimate statement above are as follows
A: Spironolactone/Hydrochlorothiazide 25 mg/25 mg film coated tablets manufactured at the
current site (Viatris) under fasting conditions.
B: Spironolactone/Hydrochlorothiazide 25 mg/25 mg film coated tablets manufactured at the
proposed site (Neolpharma) under fasting conditions.
*/
```

Appendix 3. List of Abbreviations

Abbreviation	Term
%CV	coefficient of variation
AE	adverse event
AUC_{extrap}%	the percent of AUC_{inf} based on extrapolation
AUC_{inf}	area under the plasma concentration-time profile from time zero extrapolated to infinite time
AUC_{last}	area under the plasma concentration-time profile from time zero to the time of the last quantifiable concentration
BE	bioequivalence
BLQ	below the limit of quantification
BP	blood pressure
CaPS	Clinical Data Interchange Standards Consortium and Pfizer Standards
CDK	cyclin-dependent kinase
CI	confidence interval
C_{last}	predicted plasma concentration at the last quantifiable time point from the log-linear regression analysis
C_{max}	maximum plasma concentration
CRF	case report form
CSR	clinical study report
ECG	electrocardiogram
H_1	alternative hypothesis
H_o	null hypothesis
HR	heart rate
k_{el}	elimination rate constant estimated from the log-linear regression analysis
LLQ	lower limit of quantification
ms	milliseconds
N/A	not applicable
NC	not calculated
NCA	non-compartmental analysis
ND	not done
NS	no sample
PK	pharmacokinetic(s)
PO	oral(ly)
PR	pulse rate
QRS	Combination of Q-, R- and S- wave on an electrocardiogram representing ventricular depolarization
QT	time from the start of the Q- wave to the end of T- wave, which represents time taken for ventricular depolarization and repolarization
QTc	corrected QT
QTcF	corrected QT (Fridericia method)
r^2	goodness of fit statistic from the log-linear regression
RR	the time between the start of one QRS complex and the start of the next QRS complex
SAP	statistical analysis plan
SD	standard deviation
SoA	schedule of activities
$t_{1/2}$	terminal plasma elimination half-life
TEAE	treatment emergent adverse event
T_{max}	time for C_{max}