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Statistical Analysis Plan

A Randomized, Double-blind, Placebo-controlled, Single and Multiple Ascending Dose, and Food Effect Evaluation Trial to Evaluate the Safety, Tolerability, and Pharmacokinetics of AQ280 in Healthy Subjects

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LIST OF ABBREVIATIONS

Abbreviations pertain to the statistical analysis plan (SAP) only (not the tables, figures, and listings [TFLs]).

$\%AUC_{extrap}$	percentage of area under the concentration-time curve due to extrapolation from the last quantifiable concentration to infinity
ADaM	Analysis Data Model
AE	adverse event
ANOVA	analysis of variance
AR_{AUC}	accumulation ratio based on $AUC_{0-\tau}$
AR_{Cmax}	accumulation ratio based on C_{max} during the dosing interval
AUC_{τ}	area under the concentration-time curve over the dosing interval
AUC_{0-24}	area under the concentration-time curve over the time interval 0 to 24 hours postdose
$AUC_{0-\infty}$	area under the concentration-time curve from time 0 extrapolated to infinity
$AUC_{0-tlast}$	area under the concentration-time curve from time 0 to the time of the last quantifiable concentration
BLQ	below the limit of quantification
CDISC	Clinical Data Interchange Standards Consortium
CI	confidence interval
CL/F	apparent total clearance
C_{max}	maximum observed concentration
COVID-19	coronavirus disease 2019
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
C_{trough}	concentration at the end of the dosing interval
CV	coefficient of variation
$DAUC_{\tau}$	AUC_{τ} normalized by dose administered
$DAUC_{0-24}$	AUC_{0-24} normalized by dose administered
$DAUC_{0-\infty}$	$AUC_{0-\infty}$ normalized by dose administered
$DAUC_{0-tlast}$	$AUC_{0-tlast}$ normalized by dose administered.
DMP	data management plan
ECG	electrocardiogram
eCRF	electronic case report form
$F_{rel, Cmax}$	relative bioavailability in the fed and fasted state based upon C_{max}
$F_{rel,AUC0-\infty}$	relative bioavailability in the fed and fasted state based upon $AUC_{0-\infty}$
GLSM	geometric least squares mean
ICH	International Council for/Conference on Harmonisation

ln	natural log
LR	Linearity ratio
LSM	least squares mean
λ_z	apparent terminal elimination rate constant
λ_z Lower	start of exponential fit
λ_z N	number of data points included in the log-linear regression
λ_z Span Ratio	time period over which λ_z was determined as a ratio of $t_{1/2}$
λ_z Upper	end of exponential fit
MedDRA	Medical Dictionary for Regulatory Activities
MR_{AUC}	metabolite:parent ratio based on $AUC_{0-\infty}$
MR_{Cmax}	metabolite:parent ratio based on C_{max}
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
QD	once daily
R^2-adj	adjusted coefficient for determination of exponential fit
SAP	statistical analysis plan
SD	standard deviation
SDV	source document verification
$t_{1/2}$	apparent terminal elimination half-life
TEAE	treatment-emergent adverse event
TFL	table, figure, and listing
t_{last}	time of the last quantifiable concentration
t_{max}	time of the maximum observed concentration
V_z/F	apparent volume of distribution during the terminal phase
WHODrug	World Health Organization Drug Dictionary

1. INTRODUCTION

This SAP has been developed after review of the clinical study protocol (Final Version 4.0 dated 4th October 2022) and electronic case report form (eCRF).

This SAP describes the planned analysis of the pharmacokinetic (PK), pharmacodynamic (PD), safety, and tolerability data from this study. A detailed description of the planned TFLs to be presented in the clinical study report (CSR) is provided in the accompanying TFL shells document.

In general, the analyses are based on information from the protocol, unless they have been modified by agreement with Aqilion. A limited amount of information about this study (eg, objectives, study design) is given to help the reader's interpretation.

This SAP must be finalized prior to any unblinding of study data for analysis purposes (interim or final). Additionally, the SAP and TFL shells should be finalized prior to any programming activities commencing.

This SAP supersedes any statistical considerations identified in the protocol; where considerations are substantially different, they will be so identified. If additional analyses are required to supplement the planned analyses described in this SAP, they may be performed and will be identified accordingly in the CSR. Any substantial deviations from this SAP will be agreed with Aqilion and identified in the CSR.

This SAP is written with consideration of the recommendations outlined in the International Conference on Harmonisation (ICH) E3 guideline *Structure and Content of Clinical Study Reports*, ICH E8 guideline *General Considerations for Clinical Trials*, ICH E9 guideline *Statistical Principles for Clinical Trials*.[1,2,3]

The document history is presented in Appendix 1.

2. STUDY OBJECTIVES

Table 1: Objectives and Endpoints

Objectives	Endpoints
Primary:	
• to evaluate the safety and tolerability of single and multiple ascending oral doses of AQ280, and to determine a safe therapeutic range of AQ280 in healthy subjects	• number of TEAEs per subject • clinically significant abnormalities in vital signs (systolic and diastolic blood pressure, pulse rate, and oral body temperature) • abnormal ECG (QTcF interval of >450 msec for males and >470 msec for females, or change from baseline of >30 msec) measured from Day 1 (postdose) up until 48 hours postdose in Part A and up to the follow-up visit in Part B • clinically significant changes in laboratory evaluations

Objectives	Endpoints
Secondary: • to determine the PK of AQ280 after single and multiple oral doses • to determine the PK of the AQ280 main metabolite, AQ282, after single and multiple oral doses • to determine the effect of food on the PK of AQ280 after single oral dose	Part A (SAD): • primary PK parameters derived from plasma concentration-time profile of AQ280: $AUC_{0-\infty}$, C_{max} ($AUC_{0-tlast}$ may be included as a primary PK parameter if $AUC_{0-\infty}$ cannot be calculated) • primary PK parameters derived from plasma concentration-time profile of the AQ280 main metabolite, AQ282: $AUC_{0-\infty}$, C_{max} ($AUC_{0-tlast}$ may be included as a primary PK parameter if $AUC_{0-\infty}$ cannot be calculated) • comparison of the primary PK parameters of AQ280 after single dose administration in the fasted state and in the fed state Part B (MAD): • primary PK parameters derived from plasma concentration-time profile of AQ280 on Day 1 and Day 7: AR, AUC_{τ}, C_{max} • primary PK parameters derived from plasma concentration-time profile of the AQ280 main metabolite, AQ282, on Day 1 and Day 7: AUC_{τ}, C_{max}
Exploratory: CCI	CCI

Objectives	Endpoints
[redacted]	[redacted]

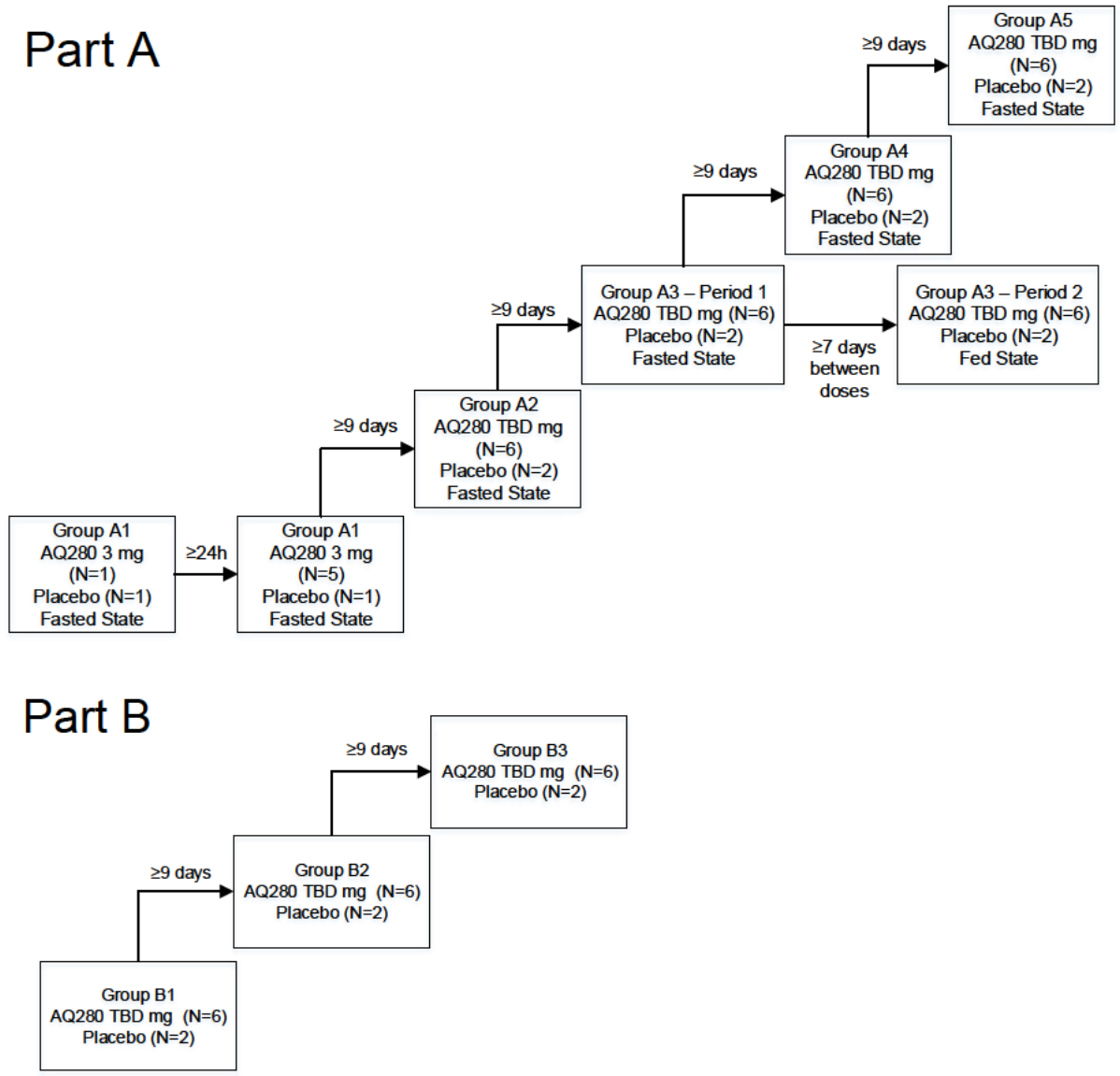
Abbreviations: AR = accumulation ratio; $AUC_{0-\infty}$ = area under the concentration-time curve from time 0 extrapolated to infinity; $AUC_{0-tlast}$ = area under the concentration-time curve from time 0 to the time of the last quantifiable concentration; AUC_{τ} = area under the concentration-time curve over a dosing interval; CL/F = apparent total clearance; C_{max} = maximum observed concentration; CCI
ECG = electrocardiogram; CCI
MAD = multiple ascending dose;
PD = pharmacodynamic; PK = pharmacokinetic(s); QTcF = QT interval corrected for heart rate using Fridericia's method; SAD = single ascending dose; $t_{1/2}$ = apparent terminal elimination half-life; TEAE = treatment-emergent adverse event; t_{max} = time of the maximum observed concentration; V_z/F = apparent volume of distribution.

3. STUDY DESIGN

3.1. Overall Study Design and Plan

This will be a Phase 1, randomized, double-blind, placebo-controlled, single and multiple ascending dose trial to evaluate the safety, tolerability, and PK of AQ280. An investigation of food effect will be included in the single dose administration part of the study. The study will be conducted in 2 parts – Part A, single ascending dose (SAD) evaluations, including food effect, and Part B, multiple ascending dose (MAD) evaluations. Schematics of the planned groups for each part are presented in Figure 1.

Figure 1: Planned Groups (Parts A and B)



Abbreviations: N = number of subjects; TBD = to be determined.

3.1.1. Part A (Single Ascending Dose)

Part A will comprise a single-dose, sequential group, escalating-dose design conducted in healthy adult male and female subjects; also incorporating a single-group, 2-period crossover arm investigating the effect of dosing AQ280 with food compared to dosing in the fasting condition. The first dose level administered is planned to be 3 mg AQ280.

A total of approximately 40 subjects are planned to be studied in 5 groups (Groups A1 to A5; with one of the groups including the food effect evaluation). In each group, 6 subjects will receive AQ280 and 2 subjects will receive placebo. The dose will be administered in the fasted state in accordance with the randomization schedule on the morning of Day 1 for all groups, including Period 1 for the food effect evaluation group (see Section 3.1.1.1 for additional food-evaluation group requirements). Each group should contain both male and

female subjects, preferably at least 2 subjects of each gender. Additional groups may be added based on the need for more evaluations or groups removed based on data obtained.

The first group in Part A will be divided into 2 cohorts, with each cohort being dosed at least 24 hours apart. The first cohort will comprise 2 subjects, with 1 subject receiving AQ280 and 1 subject receiving placebo. The second cohort will comprise 6 subjects, with 5 subjects receiving AQ280 and 1 subject receiving placebo. The 2 sentinel subjects (first cohort) will be dosed at least 24 hours before the second cohort of 6 subjects. Dosing of subjects in the second cohort will not occur if any of the dose escalation stopping criteria are met by the 2 sentinel subjects.

There will be a minimum of 9 days between dose escalations for each group.

Potential subjects will be screened to assess their eligibility to enter the study from 2 days up to 5 weeks before the dose of AQ280 or placebo. Each subject will participate in 1 group only and will reside at the study site from Day -1 (the day before dosing) to Day 3 of the treatment period, with dose administration occurring on Day 1. A safety follow-up phone call will be conducted 1 week (±2 days) after dosing.

Each eligible subject will participate in 1 treatment period only, except for the food effect group, where each subject will participate in 2 treatment periods.

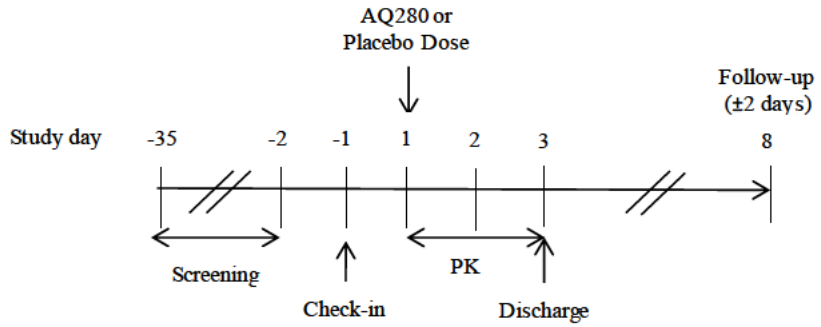
Based on the ongoing review of the safety, tolerability, and PK results, additional outpatient visits may be required.

Safety assessments will include AE reporting, vital signs, electrocardiograms (ECGs), clinical laboratory tests, and physical examination.

Blood samples to determine the PK of AQ280 and its main metabolite, AQ282, will be collected from predose up to 48 hours postdose.

An overview of the study design for Part A is shown in Figure 2.

Figure 2: Study Schematic (Part A)



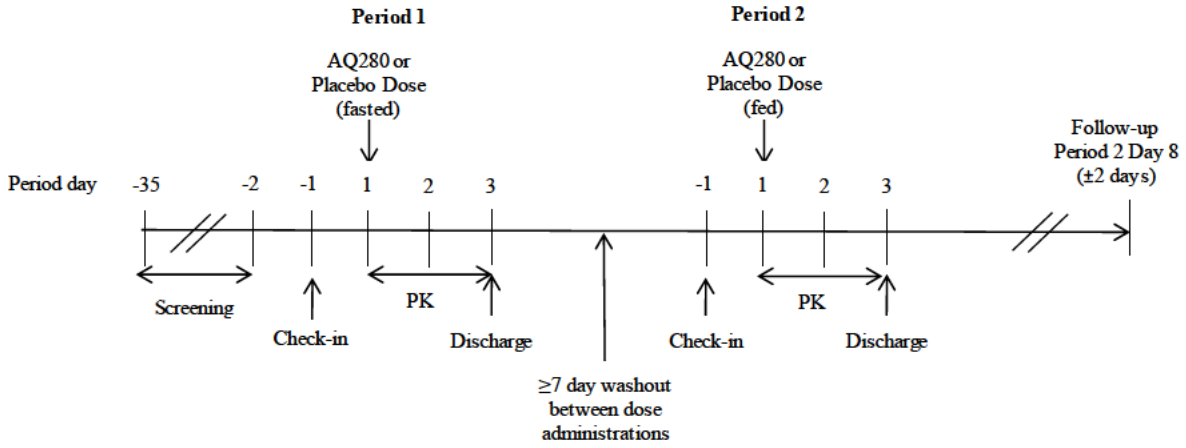
Abbreviation: PK = pharmacokinetics.

3.1.1.1. Food Effect Evaluation

Subjects in the food effect evaluation group (A3) will participate in 2 treatment periods, with the same treatment (AQ280 or placebo) administered in both periods. Period 1, Day 1 dose will be administered in the fasted state in accordance with a randomization schedule and Period 2, Day 1 dose will be given 30 minutes after starting a standard high-fat breakfast. The dose administrations in the 2 treatment periods will be separated by a washout of ≥7 days.

An overview of the study design for Part A, food effect evaluation, is shown in Figure 3.

Figure 3: Study Schematic (Part A, Food Effect Evaluation)



Abbreviation: PK = pharmacokinetics.

The total duration of study participation for each subject (from screening through safety follow-up visit) is anticipated to be approximately 6 weeks. For the food effect evaluation group, up to approximately 8 weeks, including screening and safety follow-up, is anticipated for each subject.

3.1.2. Part B (Multiple Ascending Dose)

Part B will comprise a multiple-dose, sequential group, escalating-dose design conducted in healthy adult male and female subjects. Part B may start prior to completion of all planned dose groups in Part A, at a dose equal to or less than that evaluated as safe and well tolerated in Part A.

A total of approximately 24 subjects are planned to be studied in 3 groups (Groups B1 to B3). In each group, 6 subjects will receive AQ280 and 2 subjects will receive placebo. Each group should contain both male and female subjects, preferably at least 2 subjects of each gender. Additional groups may be added based on the need for more evaluations or groups removed based on data obtained.

There will be a minimum of 9 days between dose escalations for each group.

Potential subjects will be screened to assess their eligibility to enter the study from 2 days up to 5 weeks before the first dose of AQ280 or placebo. Each subject will participate in 1 group only and reside at the study site from Days -1 to 9.

For all subjects, dosing is planned to be once daily (QD) on Days 1 to 7, inclusive, at approximately the same time each morning. The dosing regimen in Part B may be changed following review of preliminary data from groups in Part A or previous groups in Part B. The daily dose administered, when given repeatedly, will be predicted to not exceed an exposure shown to be safe and well tolerated in Part A. Dietary state will be determined based on data obtained in Part A.

A safety follow-up visit will be conducted 1 week (±3 days) after the final dose.

Based on the ongoing review of the safety, tolerability, and PK results, additional outpatient visits may be required.

Safety assessments will include AE reporting, vital signs, ECGs, clinical laboratory tests, and physical examination.

Blood samples to determine the PK of AQ280 and its main metabolite, AQ282, will be collected from predose up to 24 hours postdose on Day 1; predose on Days 3 through 6; and from predose up to 48 hours postdose on Day 7.

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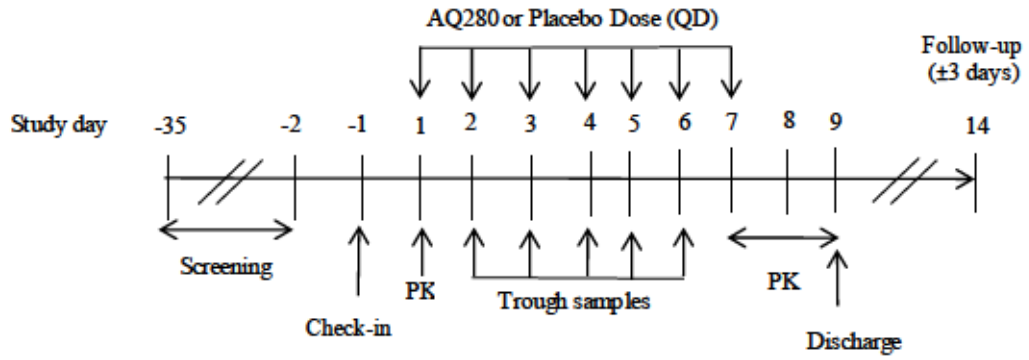
An overview of the study design for Part B is shown in Figure 4.

Figure 4: Study Schematic (Part B)



Abbreviations: PK = pharmacokinetics; QD = once daily.

The total duration of study participation for each subject (from screening through follow-up visit) is anticipated to be approximately 7 weeks.

4. SAMPLE SIZE JUSTIFICATION

No formal statistical assessment, in terms of sample size, has been conducted as this is the first time AQ280 is being administered to humans. However, the number of subjects in each

part of the present study is common in early clinical pharmacology studies and is considered sufficient to achieve the objectives of the study.

5. STUDY TREATMENTS

The study treatment names and ordering to be used in the TFLs are presented for Part A (Table 2) and Part B (Table 4). The study treatment sequence names and ordering to be used in the TFLs for Part A are presented in (Table 3).

Table 2: Presentation of Study Treatments in TFLs (Part A)

Part	Group	Study Treatment	Order in TFLs
A	A1 to A5	Placebo	1
	A1	3 mg AQ280 (Fasted)	2
	A2	XX mg AQ280 (Fasted)	3
	A3	XX mg AQ280 (Fasted)	4
		XX mg AQ280 (Fed)	5
	A4	XX mg AQ280 (Fasted)	6
	A5	XX mg AQ280 (Fasted)	7

Table 3: Presentation of Study Treatment Sequences in TFLs (Part A; Food-effect Group Only)

Group	Study Treatment Sequence	Order in TFLs
A3	Placebo (Fasted) / Placebo (Fed)	1
	XX mg AQ280 (Fasted) / XX mg AQ280 (Fed)	2

Table 4: Presentation of Study Treatments in TFLs (Part B)

Group	Study Treatment	Order in TFLs
B1 to B3	Placebo	1
B1	XX mg AQ280 QD	2
B2	XX mg AQ280 QD	3
B3	XX mg AQ280 QD	4

QD = once daily

All TFLs will be based on actual treatments (eg, if subject was assigned to receive placebo but was wrongfully dosed with active treatment they would be summarized and listed under active treatment).

The TFLs will reflect the dose levels utilized in the study, and these will be displayed in increasing order.

6. DEFINITIONS OF POPULATIONS

Any protocol deviations, including those due to coronavirus disease 2019 (COVID-19) and related restrictions (see Section 7.1.1), will be considered prior to database lock for their importance and taken into consideration when assigning subjects to populations. For all populations subjects will be analysed according to the treatment they actually received.

6.1. All Subjects Population

The all subjects population will include all subjects who signed the ICF and had any study assessment recorded in the database per the protocol.

6.2. Safety Population

The safety population will include all subjects who received at least 1 dose of study treatment (AQ280 or placebo).

6.3. Pharmacokinetic Population

The PK population will include all subjects who received at least 1 dose of active study treatment (AQ280) and have at least 1 valid PK concentration.

6.4. Pharmacodynamic Population

The PD population will include all subjects who received at least 1 dose of study treatment (AQ280 or placebo) and have at least 1 valid postdose PD assessment.

7. STATISTICAL METHODOLOGY

7.1. General

Listings will be provided for all data captured in the database, with the exception of medical history. Listings will include all subjects assigned to the all subjects population and include data up to the point of study completion or discontinuation. Subjects are generally considered to have completed the study if they complete the scheduled follow-up phone call or visit, as applicable (rather than early termination visit). Any subject who discontinues the study will be identified accordingly in the listings. Summaries and statistical analyses will include the subjects assigned to the relevant population based on data type.

Data analysis will be performed using the SAS® statistical software package Version 9.4 (or higher if a new version is issued during the study).

Analysis Data Model (ADaM) datasets will be prepared using Clinical Data Interchange Standards Consortium (CDISC) ADaM Version 2.1 (or higher if a new version is issued during the study) and CDISC ADaM Implementation Guide Version 1.1 (or higher if a new version is issued during the study). Pinnacle 21 Community Validator Version 4.0.1 (or

higher if a new version is issued during the study) will be utilized to ensure compliance with CDISC standards.

For all statistical analyses, the hypothesis testing will be 2-sided and carried out on 0.05 significance level, unless specifically stated otherwise.

Caution should be used when interpreting results from the statistical analyses conducted in this study because the sample size is not based on power calculations.

Where reference is made to 'valid' data, this refers to non-missing data which meet the predetermined criteria (eg, are not flagged for exclusion).

Where reference is made to 'all calculations', this includes, but is not limited to, summary statistics, statistical analyses, baseline derivation, and changes from baseline.

All figures will be produced on linear-linear or discrete-linear scales, as applicable, unless specifically stated otherwise.

7.1.1. Handling of Data Quality Issues Due to Coronavirus Disease 2019 and Related Restrictions

Due to COVID-19 and related restrictions, there is a high risk for impact to data integrity, with the recognized potential for:

- Missed visits, caused by, for example:
  - Subject unable to travel to site due to restrictions, the need to quarantine, or COVID-19 infection
  - Subject unwilling to go to site due to fear of COVID-19 infection
  - Site postponing subject's visit due to investigator not being available (eg, if they have been dispatched to hospital handling COVID-19 infections)
- Site unable to replenish supply of investigational product
- Incomplete data entry by sites due to limited resources to support study or no access to source documents or to eCRF
- Outstanding source document verification (SDV) due to sponsor or country restrictions on remote SDV, or no or limited access to site(s) for on-site visits
- Unanswered queries

At the time of the reporting of the study results, all protocol deviations due to COVID-19 or related restriction will be assessed for their severity and impact on the analyses. If needed, appropriate statistical methods will be applied as a mitigating action (eg, data might be categorized into 2 analysis groups, with and without COVID-19 and related restrictions

impact); however, this will exclude any imputations of the missing values. Any mitigating actions will be agreed with Aqilion in advance and identified in the CSR.

7.1.2. Calculation of the Summary Statistics

For continuous data the following rules will be applied:

- Missing values will not be imputed, unless specifically stated otherwise.
- Unrounded data will be used in the calculation of summary statistics.
- If the number of subjects with valid observations (n) <3, summary statistics will not be calculated, with the exception of n, minimum, and maximum.
- In general, as early termination data are not associated with any scheduled timepoint, they will be excluded from all calculations of summary statistics and statistical analyses. Exceptions may be made where justified.

For categorical data the following rules will be applied:

- For ordered categorical data (eg, adverse event [AE] severity), all categories between the possible minimum and maximum categories will be included, even if n = 0 for a given category.
- For non-ordered categorical data (eg, race), only those categories for which there is at least 1 subject represented will be included; unless specifically stated otherwise.
- Missing values will not be imputed, unless specifically stated otherwise. A 'missing' category will be included for any parameter for which information is missing. This will ensure that the population size totals are consistent across different parameters.

7.1.3. Triplicate Readings

For vital signs data only, where triplicate readings are taken, the median of triplicate readings will replace the separate individual triplicate readings in all calculations.

For 12-lead electrocardiogram (ECG) data only, where triplicate readings are taken, the mean of triplicate readings will replace the separate individual triplicate readings in all calculations.

In case of incomplete triplicate readings (eg, only 2 out of 3 readings were recorded), the mean and/or medians will be calculated, as appropriate, based on the number of readings available.

7.1.4. Repeat and Unscheduled Readings

For vital signs and 12-lead ECG data only, any predose value recorded in addition to the original value or a postdose value recorded within 15 minutes of the original value will be defined as a repeat value; any postdose value recorded more than 15 minutes after the original value will be defined as an unscheduled value. For all other data types (eg, laboratory

parameters), any value recorded in addition to the original value will be defined as an unscheduled value.

The original value will be replaced by the last associated repeat value in all calculations.

As unscheduled values are not associated with any scheduled timepoint, they will be excluded from all calculations, with the exception of the baseline derivation (see Section 7.1.5).

7.1.5. Definitions of Baseline and Change from Baseline

The baseline will be defined as the last value recorded prior to the first dose, for food-effect group(s) only: in each period. If the date/time of the value is incomplete or missing, it will be excluded from the baseline calculation, unless the incomplete date/time indicates the value was recorded prior to the first dose.

Individual changes from baseline will be calculated by subtracting the individual subject's baseline value from the value at the postdose timepoint.

The summary statistics for change from baseline will be derived from individual subjects' values (eg, mean change from baseline will be the mean of the individual changes from baseline for all subjects, rather than difference between the mean value at the postdose timepoint and mean value at baseline).

See Section 7.1.4 for more detail on handling repeat and unscheduled readings in the calculations. See Section 7.1.3 for more detail on handling of triplicate readings in the calculations.

7.2. Subject Disposition and Population Assignment

Subject disposition and population assignment will be listed.

A summary table by part and treatment will be provided, based on the safety population.

7.3. Screening Demographics

The screening demographics including age, sex, race, ethnicity, height, body weight, and body mass index will be listed.

A summary table by part and treatment will be provided, based on the safety population.

7.4. Prior and Concomitant Medication

Prior medication will be defined as medication that ends prior to the first dose. Concomitant medication will be defined as medication that starts during or after the first dose or starts but does not end prior to the first dose.

Prior and concomitant medications will be coded using the World Health Organization Drug Dictionary (WHODrug) Global, Format B3, Version March 2022 (or later if a new version is

issued during the study; see the data management plan [DMP] for more details). Prior and concomitant medications will be listed.

7.5. Pharmacokinetic Assessments

7.5.1. Pharmacokinetic Analysis

The following PK parameters will be determined where possible from the plasma concentrations of AQ280 and AQ282 using noncompartmental methods in validated software program Phoenix WinNonlin (Certara, Version 8.1.1 or higher):

Parameter	Units[a]	Definition
$AUC_{0\text{-}tlast}$	h*ng/mL	area under the concentration-time curve from time 0 to the time of the last quantifiable concentration (t_{last}) (Part A and Day 1 of Part B only) [b]
$AUC_{0\text{-}24}$	h*ng/mL	area under the concentration-time curve over the time interval 0 to 24 hours postdose [b]
AUC_{τ}	h*ng/mL	area under the concentration-time curve over the dosing interval (Part B only) [b]
$AUC_{0\text{-}\infty}$	h*ng/mL	area under the concentration-time curve from time 0 extrapolated to infinity [c] (Part A and Day 1 of Part B only)
$\%AUC_{extrap}$	h*ng/mL	percentage of area under the concentration-time curve due to extrapolation from the last quantifiable concentration to infinity(Part A and Day 1 of Part B only)
C_{max}	ng/mL	maximum observed concentration
t_{max}	h	time of the maximum observed concentration
t_{last}	h	time of the last quantifiable concentration
C_{trough}	ng/mL	concentration at the end of the dosing interval
$t_{1/2}$	h	apparent terminal elimination half-life
CL/F	L/h	apparent total clearance (AQ280 only)
V_z/F	L	apparent volume of distribution during the terminal phase (AQ280 only)
MR_{AUC}		metabolite:parent ratio based on $AUC_{0\text{-}\infty}$
MR_{Cmax}		metabolite:parent ratio based on C_{max}
$F_{rel,\ AUC0\text{-}\infty}$		relative bioavailability in the fed and fasted state based upon $AUC_{0\text{-}\infty}$ (Food effect group (A3) only)
$F_{rel,\ Cmax}$		relative bioavailability in the fed and fasted state based upon C_{max} (Food effect group (A3) only)
AR_{AUC}		accumulation ratio based on $AUC_{0\text{-}\tau}$ (Part B only)
AR_{Cmax}		accumulation ratio based on C_{max} during the dosing interval (Part B only)
LR		Linearity ratio
$DAUC_{0\text{-}tlast}$	h*ng/mL/mg	$AUC_{0\text{-}tlast}$ normalized by dose administered.
$DAUC_{0\text{-}24}$	h*ng/mL/mg	$AUC_{0\text{-}24}$ normalized by dose administered

$DAUC_{\tau}$	h*ng/mL/mg	AUC_{τ} normalized by dose administered (Part B only)
$DAUC_{0-\infty}$	h*ng/mL/mg	$AUC_{0-\infty}$ normalized by dose administered
DC_{max}	ng/mL/mg	C_{max} normalized by dose administered

[a] Units are based on concentration units (provided by the bioanalytical lab or preferred units for presentation of PK parameters) and dose units used in the study.
[b] The AUC will be calculated using the linear trapezoidal rule for increasing concentrations and the logarithmic rule for decreasing concentrations (linear up/log down rule).
[c] Based on the last observed quantifiable concentration
[d] Calculated by dividing the parameter by the dose (mg)

Additional PK parameters may be determined where appropriate.

Pharmacokinetic analysis will be carried out where possible using actual dose administered (mg) and actual postdose blood sampling times. If an actual time is missing, the sample concentration result will be treated as missing unless there is scientific justification to include the result using the nominal time.

The parameters C_{max}, C_{trough}, t_{last}, and t_{max} will be obtained directly from the concentration-time profiles. If C_{max} occurs at more than 1 timepoint, t_{max} will be assigned to the first occurrence of C_{max}.

The metabolite:parent ratio(s) (MR_{AUC} or MR_{Cmax}) will be calculated as follows:

$$MR_{AUC} = (AUC_{0-\infty\ [AQ282]} / MW_{[AQ282]}) / (AUC_{0-\infty\ [AQ280]} / MW_{[AQ280]}),$$

where MW is the molecular weight of each analyte

$$MR_{Cmax} = (C_{max\ [AQ282]} / MW_{[AQ282]}) / (C_{max\ [AQ280]} / MW_{[AQ280]}),$$

where MW is the molecular weight of each analyte

The molecular weights of AQ280 and AQ282 to be used in the adjustment are as follows:

CCI [redacted]

[redacted]

The parameter $AUC_{0-tlast}$ or other common partial area may be used to determine MR_{AUC} if $AUC_{0-\infty}$ cannot be reliably calculated for the majority of subjects.

The accumulation ratio(s) (AR_{AUC} and AR_{Cmax}) will be calculated as follows:

$$AR_{AUC} = AUC_{0-\tau}\ \text{Profile Day 7} / AUC_{0-\tau}\ \text{Profile Day 1}$$

$$AR_{Cmax} = C_{max}\ \text{Profile Day 7} / C_{max}\ \text{Profile Day 1}$$

The linearity ratio (LR) will be calculated as ratio of $AUC_{0-\tau}$ following multiple dosing to $AUC_{0-\infty}$ following a single dose:

$$LR = AUC_{0-\tau}\ \text{Profile Day 7} / AUC_{0-\infty}\ \text{Profile Day 1}$$

7.5.1.1. Criteria for the Calculation of Apparent Terminal Elimination Rate Constant and Half-life

The start of the terminal elimination phase for each subject will be defined by visual inspection and generally will be the first point at which there is no systematic deviation from the log-linear decline in concentrations.

The apparent terminal elimination rate constant (λ_z) will only be calculated when a reliable estimate can be obtained using at least 3 data points, preferably not including C_{max}, and the adjusted coefficient for determination of exponential fit (R^2-adj) of the regression line is ≥0.7. Parameters requiring λ_z for their calculation (eg, $AUC_{0-\infty}$, $t_{1/2}$, CL/F and V_z/F) will only be calculated if the R^2-adj value of the regression line is ≥0.7.

The following regression-related diagnostic PK parameters will be determined, when possible:

Parameter	Units	Definition
λ_z	1/h	apparent terminal elimination rate constant
λ_z Upper	h	end of exponential fit
λ_z Lower	h	start of exponential fit
λ_z N	NA	number of data points included in the log-linear regression
λ_z Span Ratio	NA	time period over which λ_z was determined as a ratio of $t_{1/2}$
R^2-adj	NA	adjusted coefficient for determination of exponential fit

Where possible, the span of time used in the determination of λ_z (ie, the difference between λ_z Upper and λ_z Lower) should be ≥2 half-lives. If the λ_z Span Ratio is <2, the robustness of the $t_{1/2}$ values will be discussed in the CSR.

7.5.1.2. Criteria for Calculation and Reporting of Area Under the Concentration-time Curve

The minimum requirement for the calculation of AUC will be the inclusion of at least 3 consecutive concentrations above the lower limit of quantification. If there are only 3 consecutive concentrations, at least 1 should follow C_{max}. An exception may be made for metabolites, where C_{max} may be the last timepoint.

If the extrapolated area is >20%, $AUC_{0-\infty}$ (and derived parameters) may be excluded from the summary statistics and statistical analysis at the discretion of the sponsor or pharmacokineticist.

If $AUC_{0-\infty}$ cannot be determined reliably for all subjects and/or dose levels, an alternative AUC measure, such as AUC to a fixed timepoint, may be used in the statistical analysis of dose proportionality/food effect.

7.5.1.3. Criteria for Handling Concentration Below the Limit of Quantification or Missing Concentrations for Pharmacokinetic Analysis

Plasma concentrations below the limit of quantification (BLQ) will be assigned a value of 0 before the first measurable concentration and thereafter BLQ concentrations will be treated as missing. The following rules apply to the specific situations defined below:

- If an entire concentration-time profile is BLQ, it will be excluded from PK analysis.
- Where 2 or more consecutive concentrations are BLQ at the end of a profile, the profile will be deemed to have terminated and any further quantifiable concentrations will be set to missing for the calculation of the PK parameters, unless they are considered to be a true characteristic of the profile of the drug.
- If a predose analyte concentration is missing, following a single dose (Part A) or Day 1 dose (Part B) it will be set to 0 by default within Phoenix WinNonlin.
- For multiple dose part of the study (Study Profile Day 7), if the analyte concentration at the end of the dosing interval (τ) is missing, this may be substituted with the predose concentration. Similarly, if the predose concentration is missing then this may be substituted with the concentration at τ.

7.5.1.4. Treatment of Outliers in Pharmacokinetic Analysis

If a value is considered to be anomalous due to being inconsistent with the expected PK profile, it may be appropriate to exclude the value from the PK analysis. However, the exclusion of any data must have strong justification and will be documented in the CSR.

Any quantifiable predose concentration value in the first treatment period will be considered anomalous and set to missing for the PK analysis. This will be set to 0 by default in Phoenix WinNonlin.

If the predose concentration is >5% of C_{max} in the second treatment period for the food effect group (A3), all PK concentration and parameter data will be excluded from the summary statistics and statistical analysis for that period.

7.5.2. Presentation of Pharmacokinetic Data

All PK concentrations and parameters will be listed.

Summary tables, arithmetic mean (+ standard deviation [SD]) figures, overlaying individual figures, and individual figures by treatment and time postdose will be provided for plasma PK concentrations. All figures will be produced on both linear-linear and linear-logarithmic scales, with the exception of figures across all days, which will be produced on the linear-linear scale only. The +SD bars will only be displayed on the linear-linear scale.

Summary tables by treatment will be provided for all PK parameters, with the exception of diagnostic regression-related PK parameters.

A subject may be excluded from the PK summary statistics and statistical analysis if the subject has an AE of vomiting that occurs at or before 2 times the median t_{max}.

If the actual time of sample collection deviates from the nominal time by more than ±10%, the plasma concentration will be flagged and excluded from the summary statistics.

Individual concentrations deemed to be anomalous will be flagged in the listings and excluded from the summary statistics.

For plasma concentration data the following rules will apply:

- Values that are BLQ will be set to 0 for the calculation of summary statistics.
- Arithmetic mean or median values that are BLQ will be presented as 0.

For PK parameters the following rule will apply:

- Geometric mean and coefficient of variation will not be calculated for t_{last} or t_{max}.

7.5.3. Pharmacokinetic Statistical Methodology

7.5.3.1. Dose Proportionality

A statistical analysis will be conducted to investigate the dose proportionality of $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$, and C_{max} for AQ280 and AQ282 on profile day 1 for Part A and $AUC_{0\text{-}\tau}$ and C_{max} for AQ280 and AQ282 on profile day 7 for Part B.

The PK parameters will be analyzed using a power model[5] that will have the following form:

$$parameter = intercept \times dose^{\wedge}slope \times random\ error$$

Using the natural log (ln) transformation,[6] a power model can be expressed as a linear regression equation:

$$ln(parameter) = intercept + slope \times ln(dose) + random\ error$$

Dose proportionality holds if the slope of the regression line is close to 1. The estimate of the slope and its 95% confidence interval will be used to quantify the degree of non-proportionality. For each PK parameter separately, a pooled estimate (across all doses) of slope, corresponding 95% confidence interval (CI), and between-subject coefficient of variation (CV) will be calculated. Figures (on the logarithmic-logarithmic scale) containing individual values, the power model line (95% CI), and the dose proportionality line (defined as the power model line with slope of 1) will be created for each PK parameter. Additionally, figures (on the logarithmic-linear scale) containing individual values and geometric means will be created for each corresponding PK parameter normalized by dose administered.

The lack of fit test will be conducted for the statistical assessment of linearity assumption, and thus appropriateness of a power model. The lack of fit model will be the same as the power model fitted, but with dose included as an additional fixed effect. The statistical

assessment will rule the linearity assumption acceptable if the diagnostic plots appear reasonable and the lack of fit 2-sided p-value >0.05 (dose effect is not significant at the 0.05 level of significance). The assessment of linearity assumption may also occur via visual examination of the figures by the pharmacokineticist. This assessment may override the statistical assessment; where this occurs, it will be detailed in the CSR.

If the assumption of linearity is ruled acceptable and the 95% CI for the slope spans 1, it will be deemed that there is no statistical basis to conclude a lack of proportionality.

If the assumption of linearity is ruled unacceptable for any PK parameter, its corresponding PK parameter normalized by dose administered will be ln-transformed and analyzed using an analysis of variance (ANOVA) model.[7] The model will include dose as a factor.

For each PK parameter separately, the geometric least squares mean (GLSM) for each dose, p-values for the overall, and pairwise dose comparisons will be calculated. Residual plots will be produced to assess the adequacy of the model(s) fitted.

Caution should be used when interpreting results from the statistical analyses conducted in this study because no adjustment for multiple comparisons will be performed.

Examples of the SAS code that will be used are as follows:

Power Model Analysis

```
proc mixed data = <data in> alpha = 0.05;
 by parcat1n parcat1 pkday paramn param;
 model lpk = ldose / cl residual ddfm = kr2;
 ods output solutionf = <data out>;
run;
```

Power Model Analysis (Between-subject Variability)

```
proc mixed data = <data in> covtest alpha = 0.05;
 by parcat1n parcat1 pkday paramn param;
 class ldose;
 model lpk = ldose / cl residual ddfm = kr2;
 ods output covparms = <data out>;
run;
(Note: Pooled Geometric CV (%) = 100*(sqrt(exp(estimate)-1)))
```

Power Model Analysis (Lack of Fit Test)

```
proc mixed data = <data in>;
 by parcat1n parcat1 pkday paramn param;
 class dose;
 model lpk = ldose dose / htype = 1 ddfm = kr2;
 ods output tests1 = <data out>;
run;
```

ANOVA Model Analysis

```
proc mixed data = <data in> alpha = 0.05;
 by parcat1n parcat1 pkday paramn param;
 class dose;
 model ldnpk = dose / cl residual ddfm = kr2;
 lsmeans dose / cl pdiff;
 ods output lsmeans = <data out>;
```

```
 ods output diffs = <data out>;
 ods output tests3 = <data out>;
run;
```

7.5.3.2. Food Effect

A statistical analysis will be conducted to investigate the food effect on the treatment by comparing XX mg AQ280 (fed) treatment to XX mg AQ280 (fasted) treatment for Part A only.

The natural log (ln)-transformed[6] $AUC_{0\text{-tlast}}$, $AUC_{t1\text{-}t2}$, $AUC_{0\text{-}\infty}$, and C_{max} for AQ280 and AQ282 on profile day 1 will be analyzed using a mixed model.[7] The model will include actual treatment as a fixed effect and subject as a random effect.

For each PK parameter separately, the least squares mean (LSM) for each treatment, difference in LSMs between the fed and fasted treatments, and corresponding 90% and 95% confidence intervals (CIs) will be calculated; these values will then be back-transformed to give the geometric least square mean (GLSM), ratio of GLSMs, and corresponding 90% and 95% CIs; 2-sided p-value will also be calculated.

Additionally, the pooled estimate (across treatments) of the within-subject coefficient of variation (CV) will be calculated, and residual plots will be produced to assess the adequacy of the model(s) fitted.

Examples of the SAS code that will be used are as follows:

Mixed Model Analysis

```
proc mixed data = <data in>;
 by parcat1n parcat1 pkday paramn param;
 class trtan usubjid;
 model lpk = trtan / cl residual ddfm = kr2;
 lsmeans trtan / cl pdiff = control('1') alpha = 0.1;
 lsmeans trtan / cl pdiff = control('1') alpha = 0.05;
 random intercept / subject = usubjid;
 ods output lsmeans = <data out>;
 ods output diffs = <data out>;
 ods output covparms = <data out>;
run;
```

7.6. Pharmacodynamic Assessments

7.6.1. Pharmacodynamic Parameters

In Parts A and B, the relative change from baseline to 48 hours after last dose will be assessed in:

C
C
I
[redacted]

[redacted]

In Part B only, the relative change from baseline to 48 hours after last dose will be assessed in:

[redacted]

The relative change from baseline will be calculated as:

$$relative\ change = \frac{value - baseline}{baseline} \times 100$$

where baseline is the last value recorded prior to the first dose.

In Part B only, the absolute change from baseline to 48 hours after last dose will be assessed in:

[redacted]

The absolute change from baseline will be calculated as:

$$absolute\ change = value - baseline$$

where baseline is the last value recorded prior to the first dose.

Assessments may be obtained from separate blood collections or from scheduled safety clinical chemistry or hematology panels, as applicable.

7.6.2. Presentation of Pharmacodynamic Data

All PD parameters, and their relative and absolute (Part B only) changes from baseline will be listed.

Summary tables and mean figures by treatment and timepoint will be provided for all PD parameters and their relative and absolute (Part B only) changes from baseline.

Values recorded as <x, ≤x, >x, or ≥x will be displayed in the listings as recorded. For the derivation of listing flags, all calculations, and presentation in the figures, <x and ≤x values will be set to half of x, whereas >x and ≥x values will be set to x.

7.6.3. Pharmacodynamic Statistical Methodology

No inferential statistical analyses are planned.

7.7. Safety and Tolerability Assessments

7.7.1. Adverse Events

All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 25.0 (or higher if a new version is issued during the study; see the DMP for more details).

A treatment-emergent adverse event (TEAE) will be defined as an AE that starts during or after the first dose, or starts prior to the first dose and increases in severity after the first dose.

A treatment-related TEAE will be defined as a TEAE with a relationship of possibly related or related to the study treatment, as determined by the investigator.

All AEs will be listed. In addition to the data recorded in the database, the listings will include derived onset time and duration. Onset time will be calculated from the time of the last associated dose for TEAEs only. Where the last associated dose is referring to the last dose received prior to the start of a TEAE.

The frequency of subjects with TEAEs and the number of TEAEs will be summarized for the following categories:

- TEAEs (overall, serious, leading to discontinuation, and leading to death) by treatment
- TEAEs by severity and treatment
- Treatment-related TEAEs (overall, serious, leading to discontinuation, and leading to death) by treatment
- Treatment-related TEAEs by severity and treatment

The frequency of subjects will be summarized separately for TEAEs and treatment-related TEAEs by the following:

- System organ class, preferred term, and treatment
- Preferred term and treatment

For the AE data the following rules will apply:

- For the derivation of treatment-emergent status (applicable to all AEs): If the start date/time of an AE is incomplete or missing, an AE will be assumed to not be a TEAE, unless the incomplete start date/time or the end date/time indicates an AE started after the first dose.
- For the derivation of treatment-related status (applicable to TEAEs only): If the study treatment relationship for a TEAE is missing, a TEAE will be assumed to not be a treatment-related TEAE.

- For the derivation of onset time (applicable to TEAEs only): If the start date/time of a TEAE is missing, onset time will not be calculated. If the start date/time of a TEAE is incomplete, where possible, the minimum possible onset time will be calculated and presented in '≥DD:HH:MM' format (eg, if the date/time of the last associated dose is 01MAY2019/08:00 and recorded start date/time of a TEAE is 03MAY2019, then the minimum possible onset time will be calculated by assuming a TEAE started at the first hour and minute of 03MAY2019 [03MAY2019/00:00], thus will be presented as onset time ≥01:16:00 in the listing). If the start date of a TEAE is the same as the date of the last associated dose but the start time of a TEAE is missing, an onset time will be presented as '≥00:00:01'. Any clock changes will be accounted for in the derivation.

- For the derivation of duration (applicable to all AEs): If the end date/time of an AE is missing, duration will not be calculated. If the start or end date/time of an AE is incomplete, where possible, the maximum possible duration will be calculated and presented in '≤DD:HH:MM' format (eg, if the start of an AE date/time is 01MAY2019/08:00 and its recorded end date/time is 03MAY2019, then the maximum possible duration will be calculated by assuming an AE ended at the last hour and minute of 03MAY2019 [03MAY2019/23:59], thus will be presented as duration ≤02:15:59 in the listing). Any clock changes will be accounted for in the derivation.

- For the calculation of TEAE summary statistics: If the severity of a TEAE is missing, that TEAE will be counted under the 'missing' category.

- For the calculation of TEAE summary statistics: If a subject experienced multiple TEAEs with the same preferred term for the same treatment, this will be counted as 1 TEAE for that treatment under the maximum severity recorded.

7.7.2. Clinical Laboratory Parameters

All clinical laboratory parameters and their changes from baseline will be listed; any value outside the clinical reference range will be flagged. Separate listings will be provided for any parameter for which there is any individual subject value outside the respective clinical reference range.

Summary tables and boxplots by treatment and timepoint will be provided for clinical chemistry, hematology, and coagulation parameters and their changes from baseline.

Shifts from baseline tables will be provided for clinical chemistry, hematology and coagulation parameters.

Values recorded as <x, ≤x, >x, or ≥x will be displayed in the listings as recorded. For the derivation of listing flags, all calculations, and presentation in the figures, <x and ≤x values will be set to half of x, whereas >x and ≥x values will be set to x.

7.7.3. Vital Signs Parameters

All vital signs parameters and their changes from baseline will be listed; any value outside the clinical reference range will be flagged.

Summary tables and boxplots by treatment and timepoint will be provided for all vital signs parameters and their changes from baseline.

Shifts from baseline tables will be provided for vital signs parameters.

7.7.4. 12-lead Electrocardiogram Parameters

All 12-lead ECG parameters and their changes from baseline will be listed; any value outside the clinical reference range will be flagged.

Summary tables and boxplots by treatment and timepoint will be provided for all 12-lead ECG parameters and their changes from baseline.

Shifts from baseline tables will be provided for 12-lead ECG parameters.

7.7.5. Other Assessments

Medical history will not be listed.

All other safety and tolerability assessments not detailed in the above sections will be listed only.

7.7.6. Safety and Tolerability Statistical Methodology

No inferential statistical analyses are planned.

8. INTERIM ANALYSES

No formal interim analyses are planned for this study.

9. SIGNIFICANT CHANGES FROM THE PROTOCOL-SPECIFIED ANALYSES

The statistical analysis for the food effect group (A3) was changed to use a mixed model which differs from the stated analysis of variance model in the protocol.

10. REFERENCES

1. ICH. ICH Harmonised Tripartite Guideline: Structure and content of clinical study reports (E3). 30 November 1995.

2. ICH. ICH Harmonised Tripartite Guideline: General considerations for clinical trials (E8). 17 July 1997.

3. ICH. ICH Harmonised Tripartite Guideline: Statistical principles for clinical trials (E9). 5 February 1998.

4. ICH. ICH Harmonised Tripartite Guideline: Addendum on estimands and sensitivity analysis in clinical trials to the guideline on statistical principles for clinical trials (E9 [R1]). 20 November 2019

5. Gough K, Hutchinson M, Keene O, et al. Assessment of dose proportionality: report from the statisticians in the pharmaceutical industry/Pharmacokinetics UK Joint Working Party. *Drug Inf J*. 1995;29(3):1039-1048.

6. Keene ON. The log transformation is special. *Stat Med*. 1995;14(8):811-819.

7. Brown H, Prescott R. *Applied Mixed Models in Medicine*. Chichester: John Wiley & Sons, 1999.

11. APPENDICES

Appendix 1: Document History

Status and Version	Date of Change	Summary/Reason for Changes
Final Version 1.0	NA	NA; the first version.

NA = not applicable