

STATISTICAL ANALYSIS PLAN

A PHASE 1, SINGLE-CENTER, REPEAT-DOSE, OPEN-LABEL, FIXED-SEQUENCE DRUG-DRUG INTERACTION STUDY OF ZX008 IN HEALTHY MALE OR FEMALE STUDY PARTICIPANTS 18 TO 55 YEARS OF AGE

PROTOCOL UP0132

Short title: Multiple-dose, Open-label, Drug-drug Interaction Study of ZX008

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LIST OF ABBREVIATIONS

ADaM	Analysis Data Model
Ae	cumulative amount of unchanged drug excreted in urine
AE	adverse event
AESI	adverse event of special interest
ANOVA	analysis of variance
ASPS	all study participants set
AUC	area under the plasma concentration-time curve from time 0 to infinity
AUC _(0-t)	area under the plasma concentration-time curve from time 0 to the time of the last quantifiable concentration
AUC _{extr}	area under the plasma concentration-time curve obtained by extrapolation
BLQ	below limit of quantification
BMI	body mass index
CDISC	Clinical Data Interchange Standards Consortium
CI	confidence interval
CL	total body clearance
CL/F	apparent total body clearance
C _{last}	last observed (quantifiable) concentration
CLR	renal clearance
C _{max}	maximum observed plasma concentration
CRO	Contract Research Organisation
CSR	clinical study report
CTR	Clinical Trial Registry
CV	coefficient of variation
CYP	cytochrome P450
DBL	database lock
DBP	diastolic blood pressure
DCM	data cleaning meeting
DDI	drug-drug interaction
DRM	data review meeting
ECG	electrocardiogram
ECHO	echocardiogram
EDV	early discontinuation visit
fe	fraction of the dose excreted unchanged in urine

FDA	US Food and Drug Administration
geoCV	geometric coefficient of variation
geoMean	geometric mean
GMR	geometric mean ratio
ICF	informed consent form
ICH	International Council for Harmonisation
IMP	investigational medicinal product
IPD	important protocol deviation
Lambda_z	first order terminal elimination rate constant
LLoQ	lower limit of quantification
MATE1	multidrug and toxin extrusion protein 1
MedDRA	Medical Dictionary for Regulatory Activities
MRAUC	metabolite to parent ratio for AUC
MRAUC _(0-t)	metabolite to parent ratio for AUC _(0-t)
MRC _{max}	metabolite to parent ratio for C _{max}
NCA	noncompartmental analysis
PASP	pulmonary artery systolic pressure
PD	protocol deviation
PK	pharmacokinetic
PKS	pharmacokinetic set
PT	preferred term
SAP	statistical analysis plan
SBP	systolic blood pressure
SD	standard deviation
SOC	system organ class
SS	safety set
t _{1/2}	terminal elimination half-life
TEAE	treatment-emergent adverse event
TFLs	tables, figures and listings
t _{last}	time of C _{last}
t _{max}	time of C _{max}
V _z	volume of distribution
V _z /F	apparent volume of distribution
WHODD	World Health Organisation Drug Dictionary

ZX008

fenfluramine hydrochloride

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STATISTICAL ANALYSIS PLAN AMENDMENT SUMMARY OF CHANGES TABLE

The SAP for UP0132 is based on the protocol dated 21 June 2024.

Document history

Document	Date	Change	Rationale
Original SAP	11 Mar 2025	Not Applicable	Original document.

1 INTRODUCTION

The purpose of this SAP is to provide all information that is necessary to perform the required statistical analysis of UP0132. It also defines the TFLs to be included in the final CSR.

If a future protocol amendment necessitates a substantial change to the statistical analysis of the study data, this SAP will be amended accordingly. In addition, if the methodology for the analysis of key endpoints must be modified or updated prior to DBL, a SAP amendment will be required. If, after DBL, additional analyses are required to supplement the planned analyses described in this SAP, these changes will be described in the CSR together with the associated rationale. Other minor changes to non-key analyses will also be documented in the CSR.

The content of this SAP is compatible with the ICH/FDA E9 Guidance document (CPMP/ICH/363/9, 1998).

1.1 Objectives and estimands/endpoints

Table 1-1: Objectives and endpoints

Objectives	Endpoints
Primary	
To evaluate the single-dose PK of 3 probe drugs (midazolam as CYP3A4 substrate, bupropion as CYP2B6 substrate, metformin as MATE1 substrate) before and after repeat doses of ZX008	The following primary noncompartmental PK endpoints will be compared for the probe drugs after a single dose administered alone (Day 1) and following 17 days of ZX008 administration (Day 22): C_{max} - Time Frame: predose and at multiple time points (up to 96 hours) postdose - AUC - Time Frame: predose and at multiple time points (up to 96 hours) postdose - AUC(0-t) - Time Frame: predose and at multiple time points (up to 96 hours) postdose with “predose” and “postdose” referring to administration of the probe drugs.
Secondary	
To evaluate the safety and tolerability of concomitant administration of the cocktail of probe drugs with ZX008 compared with the probe drugs alone	Occurrence of TEAEs - Time Frame: Day 1 to 116

To evaluate the safety and tolerability of repeat doses of ZX008	
Exploratory	
To evaluate the effect of repeat doses of ZX008 15mg bid on single-dose PK of the probe drugs	The following noncompartmental PK endpoints will be estimated for the probe drugs administered alone (Day 1) and following 17 days of ZX008 administration (Day 22): t_{max}, C_{last}, t_{last}, CL/F, V_z/F, λ_z, and $t_{1/2}$ - Time Frame: predose and at multiple time points (up to 96 hours) postdose f_e and A_e of metformin - Time Frame: predose and at multiple time points (up to 72 hours) postdose CL_R of metformin when administered alone and following 17 days of ZX008 administration - Time Frame: predose and at multiple time points (up to 72 hours) postdose with “predose” and “postdose” referring to administration of the probe drugs.
To evaluate the effect of repeat doses of ZX008 15mg bid on single-dose PK of the probe drug metabolites	The following noncompartmental PK endpoints will be estimated for the metabolites (1-hydroxymidazolam and hydroxybupropion [or [S,S]-hydroxybupropion if an enantioselective assay is selected]) when the probe drugs are administered alone (Day 1) and following 17 days of ZX008 administration (Day 22): C_{max}, $AUC(0-t)$, AUC, t_{max}, C_{last}, t_{last}, λ_z, and $t_{1/2}$ - Time Frame: predose and at multiple time points (up to 96 hours) postdose - Metabolite to parent ratio for C_{max}, $AUC(0-t)$, and AUC - Time Frame: predose and at multiple time points (up to 96 hours) postdose
To evaluate plasma concentrations of fenfluramine and norfenfluramine after repeat doses of ZX008	Trough (predose) plasma concentrations of fenfluramine and norfenfluramine - Time Frame: Day 19 to 23

To evaluate other measures of safety and tolerability of a single oral dose of the cocktail of probe drugs, repeat doses of ZX008 alone and in combination with a single oral dose of the cocktail of probe drugs	- Changes from Baseline in vital signs (pulse rate, respiratory rate, SBP, DBP, and temperature) - Time Frame: Day -35 to 116 - Physical examination findings - Time Frame: Day -35 to 116 12-lead ECG results and findings - Time Frame: Day -35 to 116 - Occurrence of clinically significant abnormal valve regurgitation and/or increased pulmonary arterial pressure - Time Frame: Day -35 to 116 - Changes from Baseline in clinical laboratory test results (hematology, clinical chemistry, and urinalysis) - Time Frame: Day -35 to 116 - Assessment of suicidality using C-SSRS - Time Frame: Screening and on Day -1, and Day 26
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Ae=cumulative amount of unchanged drug excreted in urine; AUC=area under the plasma concentration-time curve from time 0 to infinity; AUC(0-t)=area under the plasma concentration-time curve from time 0 to the time of the last quantifiable concentration; CL/F=apparent total body clearance; CL_R=renal clearance; C_{last}=last observed (quantifiable) concentration; C_{max}=maximum observed plasma concentration; C-SSRS=Columbia-Suicide Severity Rating Scale; CYP=cytochrome P450; DBP=diastolic blood pressure; ECG=electrocardiogram; fe=fraction of the dose excreted unchanged in urine; MATE1=multidrug and toxin extrusion protein 1; PK=pharmacokinetic(s); t_{1/2}=terminal elimination half-life; SBP=systolic blood pressure; TEAE=treatment-emergent adverse event; t_{last}=time of C_{last}; t_{max}=time of C_{max}; V_z/F=apparent volume of distribution; ZX008=fenfluramine hydrochloride; λ_z=first-order terminal elimination rate constant

1.2 Study design

UP0132 is a Phase 1, single-center, repeat-dose, open-label, fixed-sequence DDI study of ZX008 in 22 healthy male or female study participants aged 18 to 55 years. The study aims to determine the effect of fenfluramine and norfenfluramine following repeat doses of ZX008 on induction of CYP2B6 and CYP3A4 and inhibition of MATE1. Safety and tolerability of the probe drugs and ZX008 will be evaluated.

A sufficient number of study participants will be screened to ensure that 22 study participants enter Treatment Period 1 with the objective of having at least 18 study participants with reliable primary PK endpoints for both treatment periods. Study participants will enter a Screening Period (up to 35 days before administration of the probe drugs). All study participants will then progress through Treatment Period 1 and Treatment Period 2, comprising 6 days (Day -1 to Day 5) and 21 days (Day 6 to Day 26), respectively.

A blood sample will be collected at Screening and will be used for genotyping of CYP2B6 and CYP3A4. Participants who are otherwise qualified but elect to not provide consent for

genotyping are not eligible to participate in the study. Participants determined to have a poor metabolizer phenotype for CYP2B6 or CYP3A4 will be excluded from the study.

Eligible participants will report to the site on Day -1 (1 day before administration of the cocktail of probe drugs in Treatment Period 1) and will be housed at the site until discharge from the site on Day 26, approximately 24 hours after the final scheduled dose of ZX008 on Day 25, or after the final scheduled assessment. Participants will fast overnight for at least 8 hours before and 4 hours after probe drug administration on study Days 1 and 22. All participants will receive standard meals for breakfast, lunch, and dinner at all other times.

Each participant will receive the following treatments:

- Treatment Period 1 – a single dose of the cocktail of probe drugs on Day 1
- Treatment Period 2 – repeat oral doses of ZX008 (15mg bid) from Day 6 through Day 25 (morning dose only), and a single dose of the cocktail of probe drugs on Day 22

There will be a washout phase of 4 days (Day 2 to Day 5) between the single dose of the cocktail of probe drugs in Treatment Period 1 (Day 1) and the first dose of ZX008 in Treatment Period 2 (Day 6). Study participants will be discharged from the site 24 hours after the final planned dose of ZX008 is given on Day 25, or after early termination or withdrawal. Study participants will return to the site for a Safety Follow-Up Visit 90 days after discharge from the site, or after early termination or withdrawal.

Blood plasma and urine sampling for the determination of concentrations of probe drugs, relevant metabolites, fenfluramine, and norfenfluramine are scheduled as follows:

Treatment Period 1

- Serial blood samples for the determination of the plasma concentrations of each probe drug, 1-hydroxymidazolam, and hydroxybupropion (or [S,S]-hydroxybupropion if an enantioselective assay is utilized) will be drawn from each participant at predose, 0.25, 0.50, 1.0, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, 72, and 96 hours postdose, for a total of 17 samples per participant.
- Urine will be collected from each participant for the determination of metformin in 6-hour intervals up to 36 hours, and then in 12-hour intervals up to 72 hours after the metformin dose on Day 1.

Treatment Period 2

- Serial blood samples for the determination of the plasma concentrations of each probe drug, 1-hydroxymidazolam, and hydroxybupropion (or [S,S]-hydroxybupropion if an enantioselective assay is utilized) will be drawn from each participant at predose, 0.25, 0.50, 1.0, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, 72, and 96 hours postdose, for a total of 17 samples per participant.
- Blood samples for the determination of trough plasma concentrations of fenfluramine and norfenfluramine will be drawn from each participant before the morning dose of ZX008 on Days 19 to 23.

- Urine will be collected from each participant for the determination of metformin in 6-hour intervals up to 36 hours, and then in 12-hour intervals up to 72 hours after the metformin dose on Day 22.

The proposed plasma and urine sampling scheme are derived from literature information for similar dosing regimens and expected to capture the PK profiles (AUC) of each probe drug and the relevant metabolites.

Participants will be discharged on Day 26 after collection of the 96-hour PK blood sample, approximately 24 hours after the administration of the final dose of ZX008 in Treatment Period 2, contingent on safety and judgment of the investigator and in conjunction with the Sponsor, if deemed appropriate.

Safety and tolerability will be monitored throughout the study by AEs, examination of safety laboratory parameters, vital signs (SBP, DBP, body temperature, respiratory rate, and pulse rate), ECGs, ECHOs, and physical examinations as noted in Table 1.1 of the protocol.

2 STATISTICAL HYPOTHESES

Not applicable.

2.1 Multiplicity adjustment

Not applicable.

3 ANALYSIS SETS

The following are the defined analysis sets:

- ASPS: All study participants who sign the ICF. This analysis set includes participants who failed screening and will be used for the disposition and demographics outputs. In addition, the ASPS will be used for the listing of all AEs.
- SS: All study participants who receive at least 1 dose of any of the study treatments will be included. The SS will be used for all safety analyses.
- PKS: All study participants who receive at least 1 dose of any of the study treatments and have at least 1 quantifiable postdose PK concentration measurement of the probe drugs. Pharmacokinetic parameters will be summarized for this set.

If a study participant in the PKS is missing concentrations at individual time points or they are otherwise unobservable, the study participant will be included in the PKS, but those time points will be omitted from the PK summaries, as appropriate.

Data from participants who experience emesis during the course of the study might be excluded from the statistical analysis, depending on the time relative to the PK sampling and the frequency of the event, but the plasma concentration data will be flagged and listed.

Study participants in the PKS may not have a complete set of PK endpoints as some parameters may not be able to be determined based on the PK concentrations collected.

4 STATISTICAL ANALYSIS

4.1 General considerations

Statistical analysis and generation of tables, figures, and study participant listings will be produced by UCB. Standard reporting will use SAS version 9.4 or higher (SAS Institute, Cary, North Carolina, USA) using validated program code according to the relevant SOP. Analysis data will adhere to CDISC guidance documents for ADaM and follow the UCB interpretation. The PK noncompartmental analysis (NCA) will be performed by a designated CRO using Phoenix WinNonlin® v8.0 or higher (Certara L.P., Princeton, NJ, USA) for PK parameters estimation.

Descriptive statistics will be displayed to provide an overview of the study results, and study participant data listings are generated for relevant (as according to ICH E3) reported and/or calculated data.

Tables and figures will be presented by treatment, visit and timepoint where applicable.

For continuous endpoints, descriptive statistics will include n, mean, SD, median, minimum and maximum. GeoMean, geoSD, geoCV and 95% CIs for the geoMean will also be presented in the summaries of PK concentration data and PK parameters.

When reporting descriptive statistics, the following rules will apply in general:

- Mean (arithmetic and geometric), SD (arithmetic and geometric), CI, and median will use 1 decimal place more, or 1 significant figure more – depending on the reporting format of the original data – than the original data
- CV will be reported as a percentage to 1 decimal place
- Minimum and maximum will be reported using the same number of decimal places or significant figures as the original value
- If no participants have data at a given timepoint, then only n=0 will be presented. If n<3, then only the n, minimum, and maximum will be presented. If n=3, then only n, minimum, median, and maximum will be presented. The other descriptive statistics will be left blank
- Unless otherwise noted, all percentages will be displayed to 1 decimal place. No percentages will be displayed for zero counts and no decimal will be presented when the percentage is 100%

Time windows for PK sampling and maximum deviations from scheduled sampling times considered irrelevant for PK are documented in the protocol (Protocol Section 8.1). Time deviations considered relevant for PK will be reviewed by the PK Scientist. Any important deviations will be flagged in the PK listings and the concentrations will be excluded from the summary statistics.

Statistical model output will be placed in the ‘Documentation of Statistical Methods’ appendix of the CSR. For rules of reporting PK concentration data and PK parameters, refer to Section 6.9.

4.1.1 Analysis time points

4.1.1.1 Study periods

The study periods are as follows:

- Screening Period (Day -35 to -2)
- Treatment Period 1 (Day -1 to 5)
- Treatment Period 2 (Day 6 to 26)
- Safety Follow-up (SFU) Period (Day 27 to SFU visit)

A study participant is defined as having started a Treatment Period if they receive a single dose of the applicable study drug in that period (3-probe drug cocktail in Treatment Period 1, ZX008 alone or ZX008 in combination with 3-probe drug cocktail in Treatment Period 2).

A study participant is defined as having completed Treatment Period 1 if they have started Treatment Period 1 and are not withdrawn from the study up to and including Day 5. A study participant is defined as having completed Treatment Period 2 if they have started Treatment Period 2 and are not withdrawn from the study up to and including Day 26.

Completed study is defined as a study participant who completed all periods of the study including the safety follow-up period and did not discontinue the study early.

Study participants who discontinue the study early will be encouraged to attend the SFU visit 90 days (± 10 days) after the final administration of ZX008.

4.1.1.2 Visits variables

The visit variables will be reported in the TFLs as per [Table 4-1](#).

Table 4-1: Visit variables

Visit name in the protocol	Visit variable to be displayed in TFLs
Screening Period	
Day -35 to Day -2	Day -35 to Day -2
Treatment Period 1	
Day -1 to Day 5	Day -1, Day 1 (Baseline) to Day 5
Treatment Period 2	
Day 6 to Day 26	Day 6 to Day 26
Safety Follow-up	
Day 27 to SFU Visit	SFU Period
Day 116 ± 10 days	SFU Visit

SFU=safety follow-up.

4.1.1.3 Mapping of assessments performed at Early Withdrawal Visit

Assessments performed at EDV will be assigned to the scheduled visit after which they occurred and analyzed accordingly. Assessments performed at EDV will be flagged in the listings.

4.1.1.4 Study day for listings

Study day for an event or measurement occurring before the date of the first dose will be calculated as follows:

$$\text{Study Day} = \text{Event/Measurement Date} - \text{Date of First Dose}$$

Study day for an event or measurement occurring on the date of the first dose is 1. Study day for an event or measurement occurring on or after the date of the first dose to the date of the last visit will be calculated as follows:

$$\text{Study Day} = (\text{Event/Measurement Date} - \text{Date of First Dose}) + 1$$

There is no study day 0. Study day will not be calculated for partial dates, instead study day will be presented as '--' in the relevant participant data listing.

4.1.2 Definition of baseline values

Baseline is defined as the last available measurement (scheduled or unscheduled) prior to dosing. Baseline values for specific endpoints are outlined in [Table 4-2](#).

Table 4-2: Definition of baseline values

Endpoint	Definition of Baseline
Vital signs	Baseline is defined as the value (or mean value if measurement is taken in triplicate) at predose on Day 1. If this value is missing or multiple assessments occur, the last measurement prior to dosing will be used as Baseline.
12-lead ECG	Baseline is defined as the value (or mean value if measurement is taken in triplicate) at predose on Day 1. If this value is missing or multiple assessments occur, the last measurement prior to dosing will be used as Baseline.
Hematology, serum chemistry, urinalysis	Baseline is defined as the value on Day -1. If this value is missing or multiple assessments occur, the last measurement prior to dosing will be used as Baseline.

4.1.3 Treatment groups and combinations for analysis

The treatment groups will be displayed in TFLs as specified in [Table 4-3](#). Actual treatment will be used in all TFLs.

Table 4-3: Treatment group descriptions

Full Description	Data Display Description for Summaries	Data Display Description for Listings
Single dose of the 3-probe cocktail on Day 1	3-probe drug cocktail alone	3-probe drug cocktail alone
Repeat doses of ZX008 15mg bid from Day 6 to Day 21 and Day 23 to Day 25 (morning dose only on Day 25)	ZX008 15mg bid alone	ZX008 15mg bid alone
ZX008 15mg bid and single dose of the cocktail of probe drugs on Day 22	ZX008 15mg bid + 3-probe drug cocktail	ZX008 15mg bid + 3-probe drug cocktail

Full Description	Data Display Description for Summaries	Data Display Description for Listings
All study participants that receive at least one dose of probe drug or ZX008	All Treated Participants	Treated

4.1.4 Date Imputation

Partial dates/times, may be imputed for the following reasons:

- Classification of AEs as treatment-emergent and calculation of duration of AEs
- Classification of medications as prior or concomitant

Imputed dates will not be shown in the listings; all dates will be displayed as reported in the database.

The following rules will be applied for partial start dates/times of AEs and medications:

- If only the month and year are specified and the month and year of the first dose of study drug is not the same as the month and year of the start date then the 1st of the month will be used. If the date imputed with the first of the month is before the date of Screening, the day of Screening (also in the same year and month) will be used. If the date of Screening results in an end date that is earlier than the start date, then the 1st of the month will be used.
- If only the month and year are specified and the month and year of the first dose of study drug is the same as the month and year of the start date this should be queried and resolved prior to DBL. In the rare event they cannot be resolved, then the date of the first dose of study drug will be used. If this results in an imputed start date that is after the specified end date, then the 1st of the month will be used, or the date of Screening if this is later. If the Screening date results in an end date that is earlier than the start date, then the 1st of the month will be used. If the imputed date is the same date as the date of first dose of study drug then time will be imputed as the start time of the dosing (ie, the event will be regarded as treatment emergent or the medication will be regarded as concomitant).
- If time is missing this will be imputed as 00:00h.
- If only the year is specified, and the year of the first dose of study drug is not the same as the year of the start date then January 01 will be used.
- If only the year is specified, and the year of the first dose of study drug is the same as the year of the start date, then the date of the first dose of study drug will be used. If this results in an imputed start date that is after the specified end date, then January 01, or the date of Screening if this is later will be used (if the latter imputation results in an end date that is earlier than the start date, then January 01 will be used). If the imputed date is the date of first dose of IMP then time will be imputed as the start time of the dosing (ie, event will be regarded as treatment-emergent or the medication as concomitant).

The following rules will be applied to partial stop dates:

- If only the month and year are specified, then the last day of the month will be used.
- If only the year is specified, then December 31 of the known year will be used.

- If the stop date is completely unknown, the stop date will not be imputed.

AE duration will not be imputed for AEs with missing or partially missing dates. Missing times will be imputed as described in Table 4-4 for the calculation of duration of each AE. Adverse event duration is computed and reported in day and time format: xx d hh:mm.

Table 4-4: Calculation rules for duration of adverse events

Data availability	Onset date/time	Outcome date/time	Calculation rules
Complete data	D1/T1	D2/T2	Duration = $[(D2 - D1) * 24 + (T2 - T1)] / 24$ d
End time missing	D1/T1	D2/--	End time is substituted by time 23:59h (=23.98 in decimal format) Duration = $\leq [(D2 - D1) * 24 + (23.98 - T1)] / 24$ d
Start time missing	D1/--	D2/T2	Onset time is substituted by time 00:00h Duration = $\leq [(D2 - D1) * 24 + T2] / 24$ d
Start and end time missing	D1/--	D2/--	Duration = $\leq D2 - D1 + 1$
Start day and time missing	--/--	D2/T2	Duration = $[(D2 - D0) * 24 + (T2 - T0)] / 24$ d For a study participant in the SS, D0 and T0 are the date and time of first administration of IMP and for study participants who were randomized and not dosed, or for screen failures, D0 is the date of the Screening Visit and T0 = 00:00h
End day and time missing	D1/T1	--/--	If the stop date and time are missing, duration will not be calculated.
Start and end date missing	--/--	--/--	If the stop date and time are missing, duration will not be calculated.

d=days.

4.1.5 Multicenter studies

This is a single-center study.

4.1.6 Center pooling strategy

Not applicable.

4.1.7 Handling repeated and unscheduled measurements

Repeated and unscheduled measurements will be presented in the data listings. Repeated measurements are defined as more than 1 measurement at the same visit. The following general rules will apply to all repeated and unscheduled measurements:

- For repeated measurements that are scheduled to occur during the screening period, the latest value (which may be scheduled or unscheduled) will be used in the calculation of the descriptive statistics.
- For repeated measurements obtained at the time of the designated Baseline measurement, the latest value (which may be scheduled or unscheduled) will be defined as the Baseline provided that this occurred prior to the first dose of any study treatment.
- Unscheduled and repeated measurements will not be used in the descriptive statistics after the first dose of any study treatment is received.
- Unscheduled measurements performed at the EDV will be assigned to the subsequent visit to the one at which they occurred and analyzed accordingly.

4.2 Primary endpoints analysis

4.2.1 Pharmacokinetics

4.2.1.1 Definition of endpoint(s)

The following primary noncompartmental PK endpoints will be compared for each of the probe drugs after a single dose administered alone (Day 1) and following 17 days of ZX008 administration (Day 22):

- Maximum observed plasma concentration (C_{max})
- Area under the plasma concentration-time curve from time 0 to infinity (AUC)
- Area under the plasma concentration-time curve from time 0 to the time of the last quantifiable concentration ($AUC_{(0-t)}$)

Details of the PK parameter calculations are described in Section 6.8.

4.2.1.2 Main analytical approach

All PK endpoints will be listed and summarized using the PKS.

Pharmacokinetics endpoints will be calculated for each participant for each probe drug and their metabolites. Individual plasma concentrations of the 3 probe drugs and their metabolites over time, as well as amount of urinary excretion of metformin over time, will be summarized by treatment (probe drugs alone, probe drugs with ZX008 15mg bid) using descriptive statistics (number of observations, geometric mean, geometric SD, lower and upper 95% CI of the geometric mean, geometric CV, arithmetic mean, SD, median, minimum, and maximum).

Individual plasma concentrations of fenfluramine and norfenfluramine on Days 19, 20, 21, 22, and 23 (Treatment Period 2) will be summarized similarly. Details of the standard reporting procedures of plasma concentrations are described in Section 6.9.1.

Individual plasma concentration-actual time profiles will be displayed graphically on a linear scale and semilogarithmic scale. Plasma concentrations of the probe drugs and their metabolites

will be displayed by analyte and treatment (ie, probe drugs alone in Treatment Period 1, probe drugs with ZX008 15mg bid in Treatment Period 2). Geometric mean concentration versus scheduled (or planned) time profiles (with and without corresponding lower and upper limit of the 95% CI) will be displayed in the same way.

Spaghetti plots of the probe drugs and their metabolites will be displayed by analyte and treatment with all study participants overlaid on the same plot on a linear scale and semilogarithmic scale.

All PKS participants who have a sufficient number of bioanalytical measurements to calculate reliable estimates for the primary noncompartmental PK endpoints for either treatment (probe drugs administered alone and with ZX008 at steady state) will be included in the statistical analysis. If a participant does not have primary noncompartmental PK endpoints calculated for both treatments of a specific probe drug, they will be excluded from the main statistical analysis for that specific probe drug but will be included in a sensitivity analysis. Potential DDI effects will be evaluated by mixed effects ANOVA for logarithmically transformed (base e) primary PK endpoints of each probe drug, including treatment (administered alone and with ZX008 at steady state) as a fixed effect and study participant as a random effect. The primary statistical analysis will be repeated as a sensitivity analysis to include observations of AUC where AUC extrapolated is >20%, if any.

The least-squares mean difference between treatments (Treatment Period 2 versus Treatment Period 1) will be calculated along with 90% CI for the primary PK endpoints of midazolam, bupropion and metformin. The data will be back-transformed to provide a point estimate of the geometric mean ratio (GMR) and its 90% CI for the primary PK endpoints of midazolam, bupropion and metformin. The reference treatment will be the probe drug administered in the absence of ZX008 (Treatment Period 1).

The results obtained from the ANOVA for the primary PK parameters for each of the probe drugs will be displayed on a forest-plot. In addition, the distribution of each of the primary PK parameters of each of the probe drugs will be presented via a boxplot.

Urine PK endpoints of metformin only will be computed from the individual urine concentrations and volume of urine excreted using a noncompartmental approach.

Urine collections noted as “No Void” will be treated as zero in volume, and cumulative and derived endpoints will be included in the summary statistics. Cumulative urine PK endpoints (eg, Ae) and derived endpoints (eg, fe) after a missing sample (eg, void missing/lost, volume not recorded) will be presented with a flag alongside, but excluded from summary statistics.

4.3 Secondary endpoint(s) analysis

4.3.1 Treatment-emergent adverse events

4.3.1.1 Definition of endpoint

All AEs will be coded using MedDRA® version 27.1 and characterized as pretreatment (prior to the first dose) and treatment-emergent according to the administration of study treatments.

A TEAE is defined as any AE with a start date and time on or after the first dose of any study drug or any unresolved event already present before treatment administration that worsens in frequency or intensity following exposure to any of the study drugs. TEAEs will be assigned to

the administration of the cocktail of probe drugs alone, ZX008 alone, or the combined administration depending on the start date and time of the TEAE. TEAEs will be attributed to the most recent treatment received.

4.3.1.2 Main analytical approach

The number and percentage of participants who experience TEAEs will be summarized by MedDRA SOC, PT and treatment. Summaries of TEAEs will include the following:

- Incidence of overall TEAEs (overview including number and percentage of participants with any TEAEs, serious TEAEs, discontinuations due to TEAEs, drug-related TEAEs, severe TEAEs, TEAEs leading to death, participants meeting Hy's Law, and all deaths; event counts will also be included)
- Incidence of all TEAEs
- Incidence of TEAEs by maximum intensity
- Incidence of TEAEs by maximal relationship (to each treatment as appropriate)
- Incidence of non-serious TEAEs above reporting threshold of 5% of study participants
- Incidence of serious TEAEs by relationship
- Incidence of fatal TEAEs by relationship
- Incidence of drug-related TEAEs – overview
- Incidence of drug-related TEAEs by PT
- Incidence of serious drug-related TEAEs by PT

The AEs of special interest are detailed in Section 6.7.

Summary tables will contain counts of study participants, percentages of study participants in parentheses and the number of events where applicable. A participant who has multiple events in the same SOC and PT during a given treatment will be counted only once in the participant counts for that treatment, but all events will be included.

In summaries including relationship, the following relationships will be summarized: 'Any', 'Not related', and 'Related'. Participants who experience the same event multiple times will be included in the most related category for tabulations by maximum relationship, per treatment. Events with missing relationship will be considered as 'Related' events for summary purposes but recorded as missing in the listings.

In summaries including intensity, the following intensity categories will be summarized: 'Any', 'Mild', 'Moderate', and 'Severe'. Participants who experience the same event multiple times will be included in the most severe category for tabulations by maximum intensity. Events with missing intensity will be considered as 'Severe' events for summary purposes but recorded as missing in the listings.

Adverse event summaries will be ordered alphabetically by SOC and decreasing frequency of PT within SOC in the ZX008 15mg bid + 3-probe drug cocktail group column for tables including event counts. For tables including only number and percentage of study participants, summaries

will be ordered alphabetically by SOC and decreasing incidence of PT within SOC in the ZX008 15mg bid + 3-probe drug cocktail group column.

The following listings will be produced using the indicated analysis set:

- All AEs (ASPS) sorted by “Not dosed” and “Dosed” to allow for identification of participants who failed screening
- All related TEAEs (SS)
- AEs leading to discontinuation (SS)
- All serious TEAEs (SS)
- All severe TEAEs (SS)

All listings will be presented by study participant and treatment and will include the onset date/time and outcome date/time of the event, the event duration (derived), treatment at onset, days on dose at onset (derived), days since first dose (derived), days since most recent dose (derived), whether the AE is treatment emergent, time to onset (derived), whether the AE is serious, intensity, the pattern of event, whether the AE led to discontinuation, whether the AE is of special interest, the relationship to study medication, the action taken with study medication, any other action taken, and the AE outcome.

4.4 Exploratory endpoint(s) analysis

4.4.1 Other pharmacokinetic endpoints

The following noncompartmental PK endpoints, apart from $t_{\max}$ and t_{last} , will be listed and summarized by treatment using descriptive statistics as for the PK endpoints described in Section 4.2.1.2:

- $t_{\max}$, C_{last} , t_{last} , CL/F , V_z/F , λ_z , and $t_{1/2}$ of the probe drugs when administered alone and following 17 days of ZX008 administration, ie, in Treatment Period 1 and Treatment Period 2, respectively - Time Frame: predose and at multiple time points (up to 96 hours) postdose on Day 1 and Day 22
- $C_{\max}$, $AUC(0-t)$, AUC , $t_{\max}$, C_{last} , t_{last} , λ_z , and $t_{1/2}$ of 1-hydroxymidazolam and hydroxybupropion when probe drugs are administered alone and following 17 days of ZX008 administration - Time Frame: predose and at multiple time points (up to 96 hours) postdose on Day 1 and Day 22
- Metabolite to parent ratio for $C_{\max}$, $AUC(0-t)$, and AUC of 1-hydroxymidazolam and midazolam, and hydroxybupropion and bupropion, when probe drugs are administered alone and following 17 days of ZX008 administration - Time Frame: predose and at multiple time points (up to 96 hours) postdose on Day 1 and Day 22
- A_e and f_e for metformin when administered alone and following 17 days of ZX008 administration - Time Frame: predose and at multiple time points (up to 72 hours) postdose on Day 1 and Day 22
- Metformin CL_R , when administered alone and following 17 days of ZX008 administration - Time Frame: predose and at multiple time points (up to 72 hours) postdose on Day 1 and Day 22

with “predose” and “postdose” referring to administration of the probe drugs.

For $t_{\max}$ of each probe drug, a nonparametric analysis will be performed to assess the difference between treatments. The PK parameters $t_{\max}$ and t_{last} will be summarized using number of observations, median, minimum, and maximum for each treatment. The median difference and 90% CI will be estimated using the Hodges-Lehmann estimator, and the p-value will be obtained using the Wilcoxon signed-rank test.

Additionally, trough (predose) plasma concentrations of fenfluramine and norfenfluramine on Days 19, 20, 21, 22, and 23 in Treatment Period 2 will be summarized.

4.5 Other Safety analysis

In general, safety analyses will be presented on the SS unless stated otherwise.

4.5.1 Vital signs

Vital signs will be measured in a semi-supine position after 5 minutes rest. The following vital signs measurements will be assessed:

- Supine SBP and DBP
- Supine pulse rate
- Body temperature (oral)
- Respiratory rate

Three readings of blood pressure and pulse will be taken at least 1 minute apart. The first reading will be rejected, the second and third readings will be averaged and the average value will be recorded in the eCRF.

Vital signs observed values and changes from Baseline will be listed by study participant number and visit. The listing will also include flagging of values outside the normal ranges (specified in [Table 4-5](#)).

Vital signs measurements will be summarized by treatment, visit and time point (where applicable) including observed values and changes from Baseline.

Table 4-5: Reference ranges for vital signs

Variable	Unit	Low	High	Change criteria
Systolic blood pressure	mmHg	<90	>130	Absolute CFB >20
Diastolic blood pressure	mmHg	<60	>85	Absolute CFB >10
Pulse rate	bpm	<60	>100	Absolute CFB >20
Respiratory rate	bpm	<12	>20	None

CFB=change from baseline.

Note: For parameters where abnormalities are defined for both the actual value (eg, abnormal low; abnormal high) as well as the change value, a potentially clinically significant abnormality occurs if any single criterion is met.

4.5.2 Clinical laboratory evaluations

Listings of clinical chemistry, hematology, and urinalysis laboratory results will be produced for participants who had a value outside the normal ranges which are clinically significant. These listings will include all data for which a participant has at least one abnormal value during the study.

Clinical chemistry and hematology parameters will be summarized by treatment and time point for both absolute values and changes from Baseline. Laboratory variables will be grouped according to the laboratory function panel (e.g., lipids, endocrine) and categorized as normal, high or low, if applicable, based on the reference range supplied by the analytical laboratory.

4.5.3 Electrocardiogram

The following ECG parameters will be summarized:

- Heart rate
- PR interval
- QT interval
- QRS interval
- QTc interval (QTcF)

All single 12-lead ECG recordings should be taken with the study participant resting in the semisupine position for at least 5 minutes before the recording and at least 10 minutes prior to dosing (predose recordings). In the case of significant findings from an ECG, 2 additional ECGs will be performed. If a triplicate ECG is recorded at Baseline, the mean value will be used for the Baseline measurement.

The ECG parameter values and change from Baseline will be summarized by treatment, visit and time point.

ECG assessments will include comments on whether the tracings are normal or abnormal, rhythm, presence of arrhythmia or conduction defects, morphology, any evidence of myocardial infarction, or ST-segment, T-Wave, and U-Wave abnormalities. In addition, heart rate and measurements of the following intervals will be reported: RR interval, PR interval, QRS width, QT interval, and QTcF.

The individual measurements will be listed presented by study participant number and visit. The listing will include details of abnormal values. Abnormality criteria to be applied in the assessment of ECG values are shown in [Table 4-6](#).

A listing of all abnormal findings will also be provided.

Table 4-6: Abnormality criteria for ECG

Parameter	Unit	High
QTcF	ms	>450
Absolute CFB QTcF	ms	>60

CFB=change from Baseline, QTcF=QT interval corrected for heart rate using Fridericia's formula.

4.5.4 Physical examination

Study participants with abnormalities in the physical examination at screening will be listed including details of the abnormality and clinical significance. No summaries of physical examination are planned. Abnormalities that occur after screening will be reported as AEs.

4.5.5 Extent of exposure

Administration of treatments will be listed for all study participants in the Safety Set. The listing will include study participant ID, visit, treatment administered, the date and time of administration of each dose, the amount of treatment administered in the morning and the evening, the total daily dose and the batch number.

4.5.6 Echocardiograms

Frequency count for overall interpretation will be presented by treatment and time point of collection. Clinically significant abnormal valve regurgitation will be summarized, displaying the frequency of each valve regurgitation and the total frequency of any valve regurgitation. In addition, instances of increased pulmonary arterial pressure will be summarized. The criteria for increased PASP is displayed in [Table 4-7](#).

Table 4-7: Abnormality criteria for ECHO

Parameter	Unit	High
PASP	mmHg	>35

PASP=pulmonary artery systolic pressure

Echocardiogram results will be listed by participant and time point of collection.

4.5.7 Additional safety assessments

The C-SSRS questionnaire responses will be listed for participants who report suicidal ideation or behavior only.

Additional laboratory tests will be listed, including serum pregnancy tests, urine drug screens and CYP genotype and phenotypes. Liver function results will additionally be listed for any participant with suspected potential drug induced liver injury.

4.6 Other analyses

4.6.1 Subgroup analyses

No subgroup analyses are planned.

4.7 Interim analyses

Not applicable.

4.8 Changes to protocol-planned analyses

4.8.1 Urine collection times

According to Section 3 of the protocol, Ae and fe of metformin will be estimated predose and at multiple time points (up to 96 hours) postdose. However, according to the schedule of assessments, urine collection is scheduled up to 72 hours postdose. [Table 1-1](#) of the SAP has

been updated to state that Ae and fe of metformin will be estimated predose and at multiple time points (up to 72 hours) postdose.

4.9 Data Monitoring Committee or other review board

Not applicable.

5 SAMPLE SIZE DETERMINATION

A sufficient number of study participants will be screened to ensure that 22 study participants enter Treatment Period 1 (ie, are dosed with the cocktail of probe drugs), with the objective of having 18 study participants complete both treatment periods and have reliable primary PK endpoints for both treatment periods. It is not planned for dropouts to be replaced, but if more participants than expected withdraw from the study, study participants who are withdrawn may be replaced following a discussion between the investigator and Sponsor.

This study is not powered to formally test hypotheses for DDIs, but the number of participants included should be sufficient to provide reliable estimates of the magnitude and variability of the DDIs.

If it is expected that the GMR of AUC (or AUC(0-t) or C_{max}) for midazolam in the presence versus absence of repeat doses of ZX008 at steady state is 1.00, a sample size of 18 will provide 95% certainty that a 90% CI for the GMR of AUC (or AUC(0-t) or C_{max}) lies within the range of 0.80 to 1.25 (this range represents a precision of ± 0.223 about the difference of the means when data are logarithmically transformed [base e]). This also applies to the primary PK endpoints for bupropion and metformin. An intraparticipant coefficient of variation for AUC (or AUC(0-t) or C_{max}) of 30% for each of the probe drugs was assumed.

Data obtained from the literature for a single dose of midazolam indicate an intraparticipant CV of 25% for clearance (CL) (Kharasch, 1999). As a sensitive CYP3A4 substrate, the variability estimate for midazolam used in the sample size assessment is expected to be representative of that for bupropion IR (Preskorn, 1989) and metformin IR (Smolders, 2017). Estimates of intraparticipant variability of CL and volume of distribution (V) of midazolam are commonly used to select the sample size for pertinent DDI studies. A more conservative estimate of 30% was used for all the probe drugs in this study, to allow for the uncertainty in the estimate of the variability.

Taking into account an estimated dropout rate of 20% of study participants, the inclusion of 22 study participants entering Treatment Period 1 is expected to be adequate to meet the objectives of the study. As it is not expected that >4 participants will be withdrawn from the participation in this study, replacement of withdrawn participants is not planned.

The sample size chosen for this study is representative of similar DDI studies previously reported.

6 APPENDIX: SUPPORTING DOCUMENTATION

6.1 Appendix 1: Coding dictionaries

Medical history and AEs will be coded using the MedDRA 27.1 or later. Medications will be coded using the WHODD B3 MAR/2024. Medical procedures will not be coded.

6.2 Appendix 2: Disposition of study participants

Study participant disposition will be listed for the ASPS and will include the following information: study participant status (screen failure, completed or discontinued), date of informed consent, date of study drug administration, and the date of premature study and/or study drug termination (if applicable).

Study participant disposition will be summarized by treatment for the ASPS. The disposition table will include the reasons for screen failure, the number of study participants re-screened, reasons for discontinuation of study, the number of participants who enter Treatment Period 1 and the number of participants who complete the study.

Discontinuation due to AEs will be summarized by treatment for the ASPS. This table will include the number and percentage of participants who experience serious fatal AEs and non-fatal AEs that lead to study discontinuation. If multiple AEs are recorded as leading to discontinuation for a participant, they will be displayed in only the most severe category.

The number and percentage of study participants included in each of the analysis sets will be listed for the All Participants Set and also summarized by treatment for the SS. Percentages will be calculated based on SS for the purpose of this summary.

Disposition events will be attributed to the treatment that the participant received prior to the event and will be displayed within this treatment group in the tables.

6.3 Appendix 3: Baseline characteristics and demographics

A listing of demographics by study participant will be presented for the ASPS. This will include the year of birth, age (in years), sex, race, ethnicity, height (cm), weight (kg), and body mass index (BMI, in kg/m² to 1 decimal place).

All demographic characteristics (except for year of birth) will be summarized by treatment for the SS. The summary of age will include descriptive statistics and categorical summaries, the latter based on requirements for EudraCT reporting.

For the EudraCT reporting, the categories will include:

- 18 to <65 years
- 65 to <85 years
- ≥85 years

BMI in kg/m² is calculated based on the height (in m) and the weight (in kg) using the following formula (if height is in cm, then height will be converted to meters by dividing by 100):

$$BMI (kg/m^2) = weight (kg) / [height (m)]^2$$

The BMI will be derived and automatically reported to 1 decimal place on the eCRF.

Childbearing potential of female participants will be listed, including the method of birth control used.

Lifestyle information will also be listed including the participant's alcohol use (former, current, never), tobacco use (former, current, never), tobacco use stop date where applicable, caffeinated beverage use (former, current, never) and illicit drug use (former, never).

6.4 Appendix 4: Protocol deviations

All PDs will be reviewed at the DCM and decisions made on whether they should be considered important or not.

IPDs are deviations from the protocol which could potentially have a meaningful impact on study conduct or on either the primary or secondary outcome(s) for an individual study participant. The criteria for identifying such PDs will be defined within the PD specifications document.

All PDs will be categorized as follows:

- Inclusion/exclusion criteria deviations
- Incorrect treatment or dose administered
- Procedural non-compliance
- Prohibited concomitant medication use
- Withdrawal criteria deviation

Should a participant be mistakenly dosed despite failing the inclusion/exclusion criteria, then their safety data would need to be reported as part of the safety analysis. Participants may be excluded from other analysis sets, but this will be determined on a case-by-case basis. If the deviation is deemed to have the potential to bias the analyses for the duration of the study then the whole participant may be removed in a sensitivity analysis on the key endpoints. The removal of the participant and the rationale will be clearly documented within the relevant TFLs.

IPDs will be identified and classified by the deviation types. A listing of all IPDs will be presented for all participants in the safety set and will include the deviation type and description.

The IPDs will be tabulated using the SS and will present the deviation type and description by treatment group.

6.5 Appendix 5: Medical history

Previous medical history conditions are defined as conditions that have resolved prior to screening. Ongoing medical history conditions are defined as conditions that are ongoing at the time of screening. Medical history and ongoing medical conditions will be summarized for Safety Set by SOC and PT. The summary will include the number and percentage of study participants and will be sorted alphabetically by SOC and by descending incidence of PT within each SOC, based on the 'All Participants' column.

6.6 Appendix 6: Prior/concomitant/follow-up medications

Prior medications include any medications that started prior to the first dose of study drug on Day 1. Concomitant medications are medications taken after the first dose of study drug on Day 1. Concomitant medications will be attributed to each treatment with which they overlap. Thus, any medications occurring between Day 1 and Day 5 inclusive will be attributed to the 3-probe cocktail; medications occurring between Day 6 and Day 21 inclusive will be attributed to ZX008; medications occurring between Day 22 and Day 25 inclusive will be attributed to ZX008 + 3-probe cocktail. Safety Follow-up medications are medications occurring between the day of

discharge (Day 26) and the SFU visit. Medications may be concomitant with more than one treatment.

Prior and concomitant medications will be summarized based on the SS by treatment. Prior and concomitant medications will be summarized separately within the same table i.e., paged by prior and concomitant medications. Prior medications which continued into the study period are also classified as concomitant and are included in both summaries. The table will be sorted alphabetically by Level 1 term, alphabetical Level 3 term within Level 1 and decreasing frequency of PT in the 'All Participants' column.

All medications will be listed.

6.7 Appendix 7: AEs of special interest

Adverse events of special interest (AESI) are:

- Hy's Law
 - Potential Hy's Law, defined as $\geq 3 \times \text{ULN}$ ALT or AST with coexisting $\geq 2 \times \text{ULN}$ total bilirubin in the absence of $\geq 2 \times \text{ULN}$ ALP, with no alternative explanation for the biochemical abnormality (ie, without waiting for any additional etiologic investigations to have been concluded). Follow-up information should be reported if an alternative etiology is identified during investigation and monitoring of the participant.
- Valvular heart disease
 - any exacerbation of valvular abnormality reported on the ECHO (e.g. Mitral regurgitation changes from "absent" to "trace" or "trace" becomes "mild")
- Pulmonary arterial hypertension
 - any ECHO finding suggestive of increased pulmonary arterial pressure, including pulmonary arterial SBP above 35mmHg.

A listing of AESI will be produced for the SS.

6.8 Appendix 8: PK calculations

If calculable, pharmacokinetic parameters will be determined using non-compartmental analysis methods with Phoenix WinNonlin® v8.0 or higher (Certara L.P., Princeton, NJ, USA) based on the concentration-time data, following these guidelines:

- Actual time will be used in the calculation of all derived pharmacokinetic parameters.
- There will be no imputation of missing data.
- BLQ values at the beginning of a subject profile (i.e., before the first incidence of a measurable concentration) will be assigned to zero. Embedded BLQ values (i.e., occurring between two measurable data points) and BLQ values occurring post- $C_{\max}$ will be considered missing.

Pharmacokinetic parameters will be estimated according to the guidelines presented in the Table below (based on: Guideline on performing NCA analysis, Global Exploratory Development (GED), 2017).

Pharmacokinetic Parameter and Estimation

Parameter	Guideline for Derivation
$C_{\max}, t_{\max}$	Obtained directly from the observed concentration-time data.
$Ae_{(0-72h)}$	The cumulative amount of unchanged drug excreted in urine for metformin will be calculated as follows: $Ae_{(0-72h)} = \sum_0^{72h} C_{ur} * V_{ur}$ where C_{ur} is the concentration of unchanged drug in the sample and V_{ur} is the recorded volume of urine in the sample.
$AUC_{(0-t)}$	The AUC from time 0 (predose) to the time of the last quantifiable concentration ($AUC_{(0-t)}$) will be calculated by a combination of linear and logarithmic trapezoidal methods. Unless specifically requested and justified the linear up/log down trapezoidal method will be employed. The $AUC_{(0-t)}$ is the sum of areas up to the time of the last quantifiable sample: $AUC_{(0-t)} = \int_0^t C(t) dt$
AUC	The area under the plasma concentration-time curve from time 0 extrapolated to infinity will be calculated as follows: $AUC = AUC_{(0-t)} + \frac{C_{last}}{\lambda_z}$ where C_{last} is the last observed (quantifiable) concentration
AUC_{extr}	The percentage of AUC obtained by extrapolation will be calculated as follows: $AUC_{extr} = \frac{AUC - AUC_{(0-t)}}{AUC} * 100$ Unless otherwise determined by the PK Scientist's best knowledge and judgement, if the AUC_{extr} is great than 20%, AUC_{extr} and all dependent parameters (ie, AUC, V_z/F and CL/F) will be flagged in listings and excluded from summary tables and statistical analysis of PK parameters unless instructed otherwise by the Sponsor. The reason for exclusion will be included in the listings.
$AUC_{(0-72h)}$	The AUC from time 0 to time 72h for metformin will be calculated as follows: $AUC_{(0-72h)} = \int_0^{72h} C(t) dt$ In the case that $t_{last} < 72h$, $AUC_{(0-72h)}$ will be calculated as follows:

Parameter	Guideline for Derivation
	- If the elimination phase can be characterized, λ_z is considered valid, and %AUC_{extr} (from t_{last} to 72h) is within acceptability criteria ($\leq 20\%$), AUC_(0-72h) will be calculated by extrapolation to 72h. - If the elimination phase can be characterized but λ_z is not considered valid (R^2 adjusted < 0.8) or %AUC_{extr} is outside acceptability criteria ($> 20\%$), t_{obs} is within the allowed time window and C_{obs} is not missing or BLQ, AUC_(0-t_{obs}) will be used in place of AUC_(0-72h). If t_{obs} is outside the allowed time window, AUC_(0-72h) will be calculated by extrapolation to 72h but flagged for exclusion. - If the elimination phase cannot be characterized, t_{obs} is within the allowed time window and C_{obs} is not missing or BLQ, AUC_(0-t_{obs}) will be used in place of AUC_(0-72h), otherwise AUC_(0-72h) will be set to missing. In the case that $t_{last} > 72h$, AUC_(0-72h) will be derived by interpolation. AUC_(0-72h) values will be flagged, or flagged and excluded based on the PK Scientist's judgement based on the reliability of extrapolation or interpolation. Note: t_{obs} is the actual sampling time at planned timepoint 72h postdose. C_{obs} is the concentration at t_{obs}.
C_{last}	Obtained directly from the observed concentration versus time curve.
CL/F	CL/F = dose/AUC CL/F = dose/AUC Dose as free base (with a correction factor to convert from HCL salt to free base equivalent) will be used for the calculation of pertinent PK parameters. The salt dose for bupropion HCL and metformin HCL will be converted to the corresponding dose as free base using the following formula: Dose as free base = dose as salt * (molecular weight free base/ molecular weight salt) - The molecular weight for bupropion HCL is 276.2 g/mol - The molecular weight for bupropion is 239.74 g/mol - The molecular weight for metformin HCL is 165.63 g/mol - The molecular weight for metformin is 129.167 g/mol No conversion is needed for midazolam dose as this is already expressed as free base.

Parameter	Guideline for Derivation
CL _R	The renal clearance for metformin will be calculated as follows: $CL_R = Ae_{0-72h}/AUC_{0-72h}$
fe	The fraction of the dose excreted unchanged in urine for metformin will be calculated as follows: $fe = Ae_{(0-72h)} / \text{Dose} \times 100$ Dose as free base will be used for metformin (389.93 mg).
t _{1/2}	$t_{1/2} = \frac{\ln 2}{\lambda_z} \approx \frac{0.693}{\lambda_z}$
t _{last}	Obtained directly from the observed concentration versus time curve.
V _Z /F	$V_Z/F = (CL/F) / \lambda_z$
λ _z	Estimated slope of the linear regression of the natural logarithm of concentration versus time during the terminal phase. - The apparent terminal phase rate-constant (λ_z) will be estimated by linear regression of concentration versus time data presented in a log-linear scale. Best fit option should be used in Phoenix WinNonlin. - Data are primarily monotonically decreasing in magnitude and are representative of the actual decline in the log concentration-time curve. - Only those data points that are judged to describe the terminal log-linear decline will be used in the regression. - A minimum number of 3 data points in the terminal phase will be used in calculating λ_z with the line of regression starting at any post-C_{max} data point (C_{max} should not be part of the regression slope). Unless otherwise determined by the PK Scientist's best knowledge and judgement or if instructed by the Sponsor, if the adjusted correlation coefficient (R² adjusted) is <0.8, then λ_z and all the λ_z dependent parameters (ie, t_{1/2}, AUC, V_Z/F and CL/F) will be flagged in listings and excluded accordingly from summary tables and statistical analysis of PK parameters. The reason for exclusion will be included in the listings. - Unless otherwise determined by the PK Scientist's best knowledge and judgement, the interval used to determine λ_z should be equal or greater than 2-fold the estimated t_{1/2}. If the interval is less than 2-fold

Parameter	Guideline for Derivation
	$t_{1/2}$, λ_z together with all the derived parameters (ie, $t_{1/2}$, AUC, V_z/F and CL/F) will be flagged in the listings and either: – Excluded from the summary tables and statistical analysis of the PK parameters, or – Included and a rationale for inclusion will be presented in the analysis documentation. • If excluded, the reason for exclusion will be included in the listings.
MRC_{max}	C_{max} metabolite to parent ratio: $MRC_{max} = \frac{C_{max}(metabolite)}{C_{max}(parent)} * \frac{Molecular\ weight\ (parent)}{Molecular\ weight\ (metabolite)}^a$
MRAUC	AUC metabolite to parent ratio: $MRAUC = \frac{AUC(metabolite)}{AUC(parent)} * \frac{Molecular\ weight\ (parent)}{Molecular\ weight\ (metabolite)}^a$
$MRAUC_{(0-t)}$	AUC_(0-t) metabolite to parent ratio: $MRAUC_{(0-t)} = \frac{AUC_{(0-t)}(metabolite)}{AUC_{(0-t)}(parent)} * \frac{Molecular\ weight\ (parent)}{Molecular\ weight\ (metabolite)}^a$

^a Molecular weight (Midazolam) = 325.77 g/mol, molecular weight (1-hydroxymidazolam) = 341.77 g/mol, molecular weight (Bupriopion) = 239.74 g/mol, molecular weight (hydroxybupropion) = 255.74 g/mol, molecular weight (Metformin) = 129.167 g/mol.

6.9 Appendix 9: PK standard reporting procedures

6.9.1 PK concentrations

When reporting individual data in listings the following rules will apply:

- Concentrations below the limit of quantification should be reported as BLQ (below the limit of quantification).
- Concentrations should be listed to the same number of significant figures supplied by the bioanalytical laboratory.

When summarizing the data in tables the following rules will apply:

- To calculate descriptive statistics, BLQ values should be set to half the LLoQ value and missing values should be excluded.
- When the total number of BLQ and missing values exceeds one third of the total then only minimum and maximum should be reported for this timepoint. Other descriptive statistics should be reported as missing. The minimum should be reported as “BLQ”.
- When the mean value includes one or more replaced BLQ values then a footnote should be included to say “contains one or more BLQ value replaced by half the LLoQ value”.

- A minimum of 3 values are required to calculate summary statistics. If only 2 values are available then only the minimum and maximum should be presented; the other descriptive statistics should be presented as missing.
- If no participants have data, only n=0 will be presented. The other descriptive statistics will be left blank.
- Descriptive statistics will be reported to the same level of precision as the individual data for the minimum and maximum, and to 1 additional decimal place or 1 additional significant figure— depending on the reporting format of the original data with a maximum of 4 significant figures - for the mean (arithmetic and geometric), SD (arithmetic and geometric), CI, and median. Geometric CV will be reported as a percentage to 1 decimal place.

6.9.2 PK parameters

When reporting individual data in listings the following rules will apply:

- Individual PK parameters should be reported to 3 significant figures.
- If a parameter cannot be calculated, it should be reported as NE (not estimable i.e. if input data is missing which prevents calculation) or NC (not calculable i.e. if the data were available but the calculation was considered unreliable).

When summarizing the data in tables the following rules will apply:

- Descriptive statistics should be reported to 4 significant figures for the mean, SD, geometric mean, geometric SD, CI, and median and to 3 significant figures for the minimum and maximum. Geometric CV will be reported as a percentage to 1 decimal place.
- If at least two thirds of the study participants have a PK parameter reported then descriptive statistics will be calculated, otherwise only minimum and maximum will be reported for this PK parameter and all other descriptive statistics will be reported as NE (i.e. not estimable).
- A minimum of 3 values are required to calculate summary statistics. If only 2 values are available then only the minimum and maximum should be presented; the other descriptive statistics should be presented as missing.
- For urine data, descriptive statistics on amount excreted or cumulative amount excreted over time only will be reported. When these amounts are not estimable they should be treated as missing. Cumulative amounts should be carried forward when the concentration in the next fraction(s) is BLQ.

6.10 Appendix 10: Data transparency reporting

The following tables outlined in previous SAP sections fulfil the criteria for transparency reporting for clinicaltrials.gov and EudraCT:

- DS_T_03 Disposition and Discontinuation Reasons [ASPS] as per Section 6.2.
- DS_T_04 Discontinuation due to AEs [ASPS] as per Section 6.2.
- DM_T_01 Demographics (all age categories are mandatory) [ASPS] as per Section 6.3.
- AE_T_01 Incidence of TEAEs – Overview (mandatory, including both All Deaths and TEAE leading to Deaths) [SS] as per Section 4.3.1.2.

- AE_T_06 Incidence of Non-Serious TEAEs Above Reporting Threshold of 5% of Participants [SS] as per Section 4.3.1.2.

For small studies in populations where these events are not expected then the study team may utilize the lines from AE_T_01. The zeros in the relevant lines are sufficient for the Clinical Trial Registry (CTR) reporting. However, if an event is observed then the relevant table must be produced by CTR reporting.

- AE_T_04b Incidence of serious TEAEs by Relationship [SS]
- AE_T_04b Incidence of fatal TEAEs by Relationship [SS]

7 REFERENCES

Approval Signatures

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