STUDY CODE: NEB-RAM-01

MENARINI RICERCHE

STATISTICAL ANALYSIS PLAN (SAP)

BIOEQUIVALENCE OF NEBIVOLOL AND RAMIPRIL FOLLOWING THEIR ORAL COADMINISTRATION AS FIXED AND EXTEMPORANEOUS COMBINATION IN HEALTHY SUBJECTS

AN OPEN-LABEL, RANDOMIZED, TWO TREATMENT, THREE PERIOD, THREE SEQUENCE, SINGLE DOSE, PARTIAL REPLICATE CROSS-OVER STUDY

Sponsor: Menarini Ricerche S.p.A, Clinical Research Department, Via Sette Santi, 1; 50131 Florence, Italy

EudraCT-No.: Not applicable

Investigational medicinal Product: Nebivolol/Ramipril 5/10 mg Fixed Dose Combination (FDC); Nebivolol 5 mg + Ramipril 10 mg as extemporaneous combination (EC).

Development Phase: Phase 1, Bioequivalence study

Indication: Not applicable, healthy male and female subjects

Date of First Subject In: 26/06/23

Date of Last Subject Out: 16/10/23

SAP Version and date: 30/10/23 Version 1.0

Protocol Version and date: 1.0 28/03/2023

SIGNATURE PAGE

I have read this report and confirm that to the best of my knowledge it accurately describes the planned statistical analyses of the study.

Author	Giovanni Marino Merlo	Giovanni Marino Merlo	30/10/23
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	Global Biostat & DM Director	Signature	Date

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1. Version History

Version	Author	Description for Revision
0.1	Giovanni Marino Merlo	This is the first issue of this document
0.1_CROSSrev	Giovanni Marino Merlo	Document updated based on CROSS revision
0.1_CROSS_MEN_rev	Giovanni Marino Merlo	Document updated based on Menarini study team revision
1.0	Giovanni Marino Merlo	This is the first official version of this document

2. List of abbreviations

ABBREVIATION	DEFINITION
ADRs	Adverse Drug Reactions
AE	Adverse Event
ACS	Abnormal clinically significant
ANCS	Abnormal not clinically significant
ANOVA	Analysis of variance
AUC	Area Under the plasma Concentration time-curve
$AUC_{(0-\infty)}$	Area Under the plasma Concentration time-curve from time 0 until infinity
$AUC_{(0-t)}$	Area Under the plasma Concentration time-curve from time 0 until the last quantifiable concentration
BLOQ	Below Limit of Quantification
BMI	Body Mass Index
BP	Blood Pressure
CA	Competent Authority
CI	Confidence Interval
C_{last}	Last quantifiable plasma drug Concentration
C_{max}	Peak Plasma Concentration
Covid-19	Corona Virus Disease 19
CRF	Case Report Form
CRO	Clinical Research Organization
CT	Clinical Trial
CTIS	Clinical Trial Information System
CTM	Clinical Trial Medication
CV%	Coefficient of Variation
CYP	Cytochrome P450
DBP	Diastolic Blood Pressure

DPO	Data Protection Officer
DSUR	Development Safety Update Report
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EMA	European Medicines Agency
EOS	End of Study Visit
EC	Extemporaneous
FDC	Fixed-Dose Combination
GCP	Good Clinical Practice
GMR	Geometric mean ratio
HbcAb	Hepatitis B core antigen
HbsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
LLOQ	Lower Limit of Quantification
LSO	Last subject completing the last visit
MSC	Member States concerned
NEB	Nebivolol
NSAE	Non-Serious Adverse Events
PCR	Polymerase chain reaction
PI	Principal Investigator
PK	Pharmacokinetic
PR	Pulse Rate
RAM	Ramipril

RMS	Reporting Member State
SAR	Serious Adverse Event
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SBP	Systolic Blood Pressure
SD	Standard Deviation
SDSM	Study Drug Safety Manager
SDSU	Study Drug Safety Unit
SmPC	Summary of Product Characteristics
SOC	System Organ Class
SUSAR	Serious Unexpected Adverse Event
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Source Data
SOP	Standard Operating Procedure
SUSAR	Serious Unexpected Adverse Drug Reaction
TEAE	Treatment-Emergent Adverse Event
TMF	Trial Master File
β-HCG	Beta-subunit of Human Chorionic Gonadotropin

3. Introduction

The purpose of this document is to provide further details about the statistical analysis methods specified in the final study protocol Version 1.0 dated 28th March 2023. The SAP follows the principles of the guidelines ICH Topic E3 and E9 regarding the structure and content of clinical study reports and regarding statistical principles for clinical trials.

Sponsor will be in charge to produce the statistical tables and listings as described in the sections 12 and 13 of this document.

3.1.Changes from study protocol

No major changes from the protocol-planned analysis have been performed. One minor change is made to the statistical model for assessing the bioequivalence (see section 12.3.3), where the subject (sequence) term is treated as random effect instead of fixed effect as stated in the study protocol.

4. Study overview

The treatment with fixed combinations for cardiovascular diseases is common as they are expected to improve therapeutic compliance over the same drugs administered separately, as outlined in the EMA guideline on clinical investigation of medicinal products in the treatment of hypertension. (3)

Menarini Ricerche is developing a fixed dose combination of Nebivolol and Ramipril for the substitution therapy in hypertensive patients whose blood pressure is adequately controlled on Nebivolol 5 mg and Ramipril 10 mg given concurrently.

In this bioequivalence study the fixed dose combinations (FDC) of Nebivolol 5 mg and Ramipril 10 mg (Nebivolol/Ramipril 5/10 mg) administered as one oral film-coated tablet represent the Test versus the Reference represented by extemporaneous co-administration of the EU authorised products Nebivolol 5 mg tablet plus Ramipril 10 mg tablet.

Nebivolol is a potent and selective ß1-adrenergic antagonist that also exhibits nitric oxide (NO)-mediated vasodilatory effects. It is a 50:50 racemic mixture of the enantiomeric pair (+)-Nebivolol and (-)-Nebivolol. The drug is devoid of intrinsic sympathomimetic activity.

Nebivolol metabolism involves the CYP2D6 isoenzyme. Following its oral administration in man, peak plasma concentrations are reached around 1-hour post-dosing. In extensive CYP2D6 metabolizers, the drug is characterized by an elimination half-life of around 10-12 hours, this value being longer (up to 3-5 times) in poor metabolizers. (4), (5)

Nebivolol was first registered in the Netherlands in October 1995 for the treatment of hypertension and is now registered in many European and Extra-European countries.

Nebivolol is available on the market as tablets containing 5 mg of active substance (Nebilet®) and is currently indicated at the dose of one tablet 5 mg/day in the treatment of essential hypertension as

well as stable mild and moderate chronic heart failure in addition to standard therapies in elderly patients ≥ 70 years. Main product characteristics of Nebivolol are summarised in the reference SmPC.

Ramipril is a potent and long-acting inhibitor of the angiotensin-converting enzyme (ACE). Ramipril is converted in vivo to its active metabolite ramiprilat. Ramiprilat inhibits the enzyme ACE. In plasma and tissue this enzyme catalyses the conversion of angiotensin I to the active vasoconstrictor substance angiotensin II, as well as the breakdown of the active vasodilator bradykinin. Reduced angiotensin II formation and inhibition of bradykinin breakdown lead to vasodilation.

Following oral administration, ramipril is rapidly absorbed from the gastrointestinal tract. Peak plasma concentrations of ramipril are reached within one hour. Based on urinary recovery, the extent of absorption is at least 56 % and is not significantly influenced by the presence of food in the gastrointestinal tract. The bioavailability of the active metabolite ramiprilat after oral administration is 45 %. Peak plasma concentrations of ramiprilat, the sole active metabolite of ramipril, are reached 2-4 hours after ramipril intake. Steady state plasma concentrations of ramiprilat after once daily dosing with the usual doses of ramipril are reached by about the fourth day of treatment.

Since angiotensin II also stimulates the release of aldosterone, ramiprilat causes a reduction in aldosterone secretion. The average response to ACE inhibitor monotherapy was lower in black (Afro-Caribbean) hypertensive patients (usually a low-renin hypertensive population) than in non-black patients.

Ramipril has been authorised in the EU since 1989, first in France and then in many other European and Extra-European countries. Ramipril is available on the market as tablets of 1.25 mg, 2.5 mg, 5 mg and 10 mg strengths and is currently indicated for the treatment of essential hypertension as well as treatment of symptomatic heart failure and renal disease (glomerular diabetic and non-diabetic nephropathy).

Main product information is summarised in the Summary of Product Characteristics (SmPC) of Delix protect®, Cardace®, Triatec® and Tritace® (2.5, 5 and 10 mg).

4.1.Study objectives

The primary objective is to evaluate the bioequivalence of the NEB/RAM 5/10 mg FDC (Test) versus NEB 5 mg + RAM 10 mg EC (Reference).

5. Investigational plan

5.1.Study configuration and structure

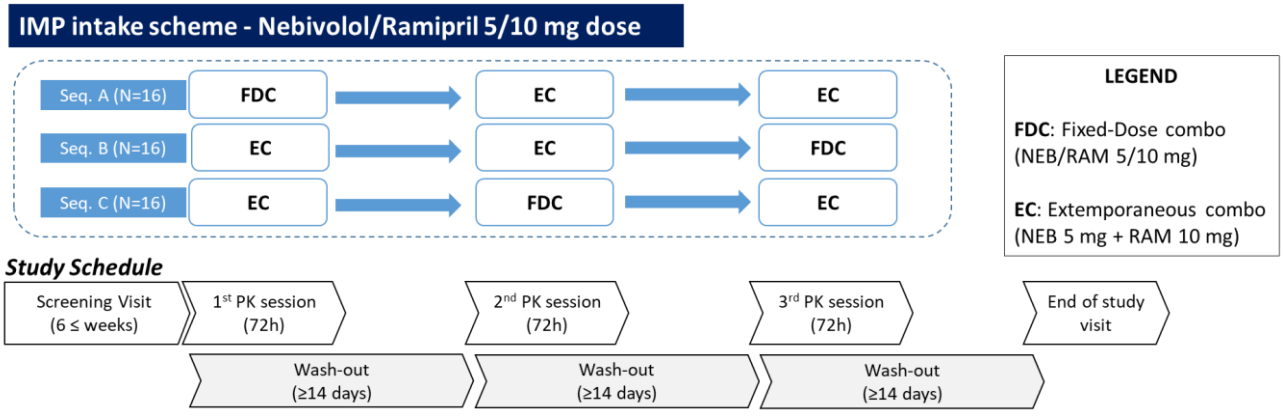
This will be an open label, randomized, two treatment, three period, three sequence, single dose partial replicate cross-over study to assess the bioequivalence of the Nebivolol/Ramipril 5/10 mg FDC (Test) versus Nebivolol 5 mg and Ramipril 10 mg administered as extemporaneous combination (Reference).

The study population will include 54 healthy males and females (18 subjects per each randomized sequence), aged 18 to 60 years inclusive, who successfully pass the Screening procedures and will receive single doses of Test and Reference formulations according to the assigned randomized sequence A, B or C, as described in the schematic study design in the section 5.2.

Subjects will undergo a Screening (to be performed within overall 4 weeks prior to 1st study PK session), three PK sessions with one single dose administration of the Test Formulation in one out of the 3 PK sessions and one single dose of the Reference Formulation in two of the 3 PK sessions as per sequence assigned by randomization. Each PK session is separated by a minimum of a 14-day period between each IMP intake. In each PK session, blood sampling for PK plasma assessment will be taken at predefined time points up to 72 hours (h) post-dose. An End of Study Visit will be performed 10-12 days after last treatment administration, resulting in an expected individual overall clinical study duration of about 10 weeks. In case of any serious adverse (drug) reaction (SAR), subject enrollment and treatment administration will be put on-hold or stopped. The study will be resumed only after the case has been discussed and a decision on how to proceed has been taken by the Sponsor.

Ramipril is reported to have an intra-subject variability above 30% for C_{max} and AUC in literature, thus falling in the definition of highly variable drug product (HVDP)[2]. In these cases, the EMA guideline on the investigation of Bioequivalence suggests to carry out a replicate study design (either a 4-period, full-replicate design or a 3-period, partial-replicate design) in order to evaluate the intra-subject variability of the reference formulation and use a scaled-average bioequivalence approach. Such design allows to widen the acceptance criteria for C_{max} to a maximum of 69.84 – 143.19% based upon the intra-subject variability of the reference formulation observed in the bioequivalence study. Details regarding the calculation of the widened acceptance limits can be found in section 12.3.3[3].

5.2. Schematic study design



Treatment sequence A, B and C will be allocated to eligible subjects who will be randomized at the CROSS RESEARCH Phase 1 Unit.

A formal safety data review meeting with Sponsor's Team and the Principal Investigators (PI) is required only in case of severe or serious adverse events.

5.3.Study flow chart

Procedures	**Screening**	**1st + 2nd + 3rd PK sessions (sessions separated by a minimum 14-day wash-out period between dosing)**																			**End of Study visit**
	≤4 weeks prior to 1st PK Session*	T+0 [1, #]	T+5'	T+15'	T+30'	T+45'	T+1h	T+1.25h	T+1.5h	T+2h	T+2.5h	T+3h	T+3.5h	T+4h	T+6h	T+8h	T+10h	T+12h	T+24h	T+48h	T+72h	**10-12 days after last dose**
Informed consent	X																					
Incl./Excl. criteria	X	X																				
Demography data	X																					
Medical history	X																					
Genotyping for CYP2D6	X																					
Physical examination	X [2]	X																				X
Haematology, biochemistry, Urinalysis	X																					X
Pregnancy test (β-HCG)[9]	X	X																				X
Serology (HIV, HBV, HCV)	X																					
Drugs of abuse [3] + alcohol test + cotinine urine test	X	X																				
Vital signs + 12-lead ECG[4]	X	X							X						X			X	X			X
Check of study restrictions		X																				
Randomisation		X[5]																				
Study Treatment Administration [6]		X																				
Nebivolol and Ramiprilat PK sample collection[7]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	

Ramipril PK sample collection[7]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Prior and Concomitant medications	X		→																		
AE recording	X[8]	X[8]	→																		
Residence in Phase I unit [&]		→																			

[1] T+0 corresponds to time of Study Treatment Administration, all other assessments at this time point to be completed prior to dosing.
[2] At Screening, physical examination also includes body weight and height.
[3] Drugs of abuse urine test cocaine and metabolites (COC 300), Amphetamine (AMP 500), Methamphetamine (MET 500), Marijuana (including Cannabinoids THC) (THC 50), Opiates (including Heroin Morphine and metabolites) (MOP 300), Methylenedioxymethamphetamine Ecstasy (MDMA 500), Methadone (MTD 300).
[4] Vital signs include supine SBP and DBP, PR, RR, Frontal Body Temperature (°C). At Screening only, orthostatic BP is also included. Prior to dosing, Vital signs and ECG should be performed within 1 hour before dosing; Post-dose assessments are limited to SBP, DBP, PR and ECG to be performed within 30 minutes before the PK sampling.
[5] Prior to dosing of the 1st PK session only.
[6] One out of two study treatments as per randomized sequence.
[7] A time window for blood sample collection is allowed as follows: ± 2 minutes for blood sampling from T+15' to T+4h; ± 5 minutes for blood sampling from T+5h to T+24h; ± 30 minutes for blood sampling at T+48h and T+72h.
[8] At screening and pre-dose (for 1st PK session only), to record also any clinical event, not associated to a drug intake, prior to IMP administration that occurs for the first time or worsens after signing the informed consent
[9] On serum at screening and on urine at each Admission to the clinical centre

[*] NOTE: Screening procedures shall start within 4 weeks prior to 1st PK study session (4 weeks also for genotyping). ALL screening procedures should be completed, and results made available prior to randomization/start of the first PK study session.
[#] NOTE: T+0 study procedures might be completed the day prior to dosing, the exception being baseline vital signs, ECG and PK sampling that should be done immediately before dosing.
[$] NOTE: re-Screening is allowed provided that the subject has not already been randomized.
[&] NOTE: Residence starts the evening before dosing and ends 72 h post dosing (each PK session). Subjects will be tested for SARS-CoV-2 infection by PCR/rapid antigen tests at screening and at each Admission to the Phase I Unit

5.4. Study Endpoints

5.4.1. Primary PK endpoints

Area Under the plasma concentration-time Curve (AUC) from time zero to the last quantifiable time point ($AUC_{(0-t)}$) and maximum plasma concentration (C_{max}) of NEB and RAM when administered as FDC film-coated tablet (Test) and as EC tablets (Reference formulation).

5.4.2. Secondary PK endpoints

- Relevant secondary standard pharmacokinetic parameters of NEB and RAM such as AUC from time zero to infinity (AUC(0-∞)), AUC from time zero to 72h (AUC(0-72)) for NEB only, plasma terminal half-life (t1/2), terminal elimination rate constant (λz), residual area (%AUCextrap), time to maximum plasma concentration (tmax), last quantifiable plasma drug concentration (Clast) and time corresponding to Clast (tlast) when NEB and RAM are administered as Test and Reference formulations.

5.4.3. Exploratory PK endpoints

- AUC from time zero to 72h (AUC(0-72)), tmax, λz, t1/2, Cmax, Clast and tlast of Ramiprilat when NEB and RAM are administered as Test and Reference formulations.

Other PK parameters for all the analytes can be derived if considered appropriate at the time of the analysis.

5.4.4.Safety endpoints

Incidence, intensity (severity), seriousness and treatment causality of Treatment Emergent Adverse Events (TEAEs, i.e. AEs that occur after the first study drug intake).

Changes in laboratory safety parameters, vital signs and 12-lead ECG will be compared versus baseline.

6. General specifications

6.1.Data validation

Medidata Rave EDC ® 2023.1.4 or subsequent version will be used as Electronic Data Capture system for data entry, by site personnel and for data cleaning and data locking by the Menarini Ricerche Data Management team. The eCRF data are elaborated to create the SDTM and ADaM CDISC standard datasets.

6.2.Computer system and software used

The software used for all summary statistics and statistical analyses will be SAS ® 9.04.01 or higher (SAS Institute, Inc.). All tables and listings will be produced using PROC REPORT or procedure specific output displays using output delivery system (ODS). The summary tables and listings will use SAS monospace font of size 6. The default page type will be A4 and the default page orientation will be landscape.

6.3.Coding systems

6.3.1. Clinical Terms

Concomitant diseases, medical procedures, and Adverse Events will be coded with MedDRA version 25.1 or subsequent.

6.3.2. Drugs

Drugs will be coded with WHO (ATC coding system) Drug version 202209 or subsequent.

6.3.3. Classification criteria

NA

6.4.Report type, language, format

The statistical output will be in word and .pdf format and in English language.

Dates will be presented with the DDMMMYYYY format.

Counts and percentages: xxx (xx.xx%)

Counts and percentages also including the count of the event of interest: xxx |xxx (xx.xx%)

Descriptive statistics:	N	xxx
	Mean	xxx.xx
	Median	xxx.xx
	SD	xxx.xxx
	Minimum	same precision as individual value
	Maximum	same precision as individual value
	Q1	xxx.xx (if present)
	Q3	xxx.xx (if present)

Character values will be left aligned.

6.5.Standard Operating Procedures (SOPs) to be followed

Code	Title
MR-GCS-DMST-201_SOP	Delegation of duties for Data Management and Biostatistics study activities
MR-GCS-DMST-208_SOP	Generation and Management of Randomization List
MR-GCS-DMST-210_SOP	Statistical Analysis Plan (SAP)
MR-GCS-DMST-210.1_WI	Sample Size Calculation

MR-GCS-DMST-211_SOP	Statistical Programs Writing
MR-GCS-DMST-211.1_WI	SDTM programming
MR-GCS-DMST-211.2_WI	ADaM programming
MR-GCS-DMST-211.3_WI	TLF programming
MR-GCS-DMST-212_SOP	SAS Use and Management

6.6.Data Transfer Agreements

Data not directly entered in eCRF are based on specific Data Transfer Agreements (DTA). In particular in the NEB-RAM-01 study the data that is not directly captured in the EDC system are the PK concentration results produced by the Analytical Laboratory Anapharm Europe, S.L.U..

For every activity performed by a laboratory, the relative procedures regarding collection, shipment and/or retention of samples will be detailed in the corresponding laboratory manual. The transfer of data to the Sponsor will be regulated with a dedicated Data Transfer Agreement (DTA).

Analytes concentrations will be provided according to the specifications defined in the data transfer agreement between the CRO and the sponsor. The transfer of the PK concentration results is performed as per “DATA TRANSFER AGREEMENT PK Samples Tracking and Concentrations NEB-RAM-01” version 1.0 dated 03 October 2023.

The transfer of PK analysis results between the Sponsor and CROSS Research S.A is conducted following the specifications reported in the “DATA TRANSFER AGREEMENT PK Analysis NEB-RAM-01” version 1.0 dated 16 October 2023.

Results of the CYP2D6 analysis will be provided according to the specifications defined in the data transfer agreement between the analytical laboratory and the site.

For the details regarding DTAs and the management of the received data, please refer to the Clinical Data Management Plan.

7. Definitions and general methodology

7.1.Data quality assurance

All tables, figures, and data listings generated by Menarini Ricerche will be independently checked for consistency, integrity and in accordance with internal standard procedures.

7.2.General considerations and key definitions

7.2.1. General considerations

Study day is defined as the number of days from the date of first dose of study drug to the event/visit date. For dates equal to or later than the first dose of study drug, study day is calculated as follows. If the event happens after the first dose, it will be:

Study Day = Event or Visit Date – First Dose Date + 1

Instead, if the event happens before the first dose, the formula that will be used will be:

Study Day = Event or Visit Date - First Dose Date

Consequently, the day immediately prior to the first dose date is assigned as Day -1. The study day of the first dose of study drug is assigned as Day 1.

7.2.2. Key definitions

Baseline values

The baseline value of an assessment is defined as the last value measured before any intake of the study drugs.

Adverse Events (AEs)

Any untoward medical occurrence in a subject or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether considered related to the medicinal product.

Treatment-Emergent Adverse Events (TEAEs)

A Treatment-Emergent Adverse Event (TEAE) stands for an AE that occurs for the first time or that worsens in terms of seriousness or severity after the first study drug intake.

Drug Relationship

The relationship between an AE and study drugs will be judged according to the following categories:

Certain: The AE occurs in a plausible time relation to the administration of the drug and cannot be explained by a concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary.

Probable: The AE occurs in a reasonable time relation to the administration of the drug, it is unlikely to be attributed to a concurrent disease or other drugs or chemicals and it follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information (AE reappearance after drug reintroduction) is not required to fulfil this definition.

Possible: The AE occurs with a reasonable time relation to the administration of the drug, but it could also be explained by a concurrent disease or other drugs or chemicals. Information on drug withdrawal (dechallenge) may be lacking or unclear.

Unassessable: The relationship cannot be judged because the information is insufficient or contradictory and cannot be supplemented or verified.

Unlikely: A causal relationship cannot be definitively ruled out, but other drugs, chemicals, or underlying disease provide plausible explanations and/or the temporal relation to the administration of the drug makes a causal relation improbable.

Not Related: Any of the following are present:

- existence of a clear alternative explanation, and/or;
- unreasonable temporal relationship between drug and event, and/or;
- non-plausibility.

Adverse Drug Reactions (ADRs)

An ADR is any untoward and unintended response to an Investigational Medicinal Product (IMP) related to any dose administered.

The definition implies a reasonable possibility of a causal relationship between the event and the IMP. This means that there are facts (evidence) or arguments to suggest a causal relationship.

An ADR is considered any AE for which the relationship is considered as:

1. Certain
2. Probable
3. Possible

4. Unassessable

An AE is not considered as ADR when the relationship is judged as:

5. Unlikely
6. Not related

Seriousness

An AE/ADR is considered serious when:

- results in death;
- is life-threatening;

NOTE: Life-threatening is considered any AE in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

- requires inpatient hospitalisation or prolongation of existing hospitalisation;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is another medically important condition that may jeopardise the subject or may require intervention to prevent one of the outcomes listed above. Any suspected transmission of an infectious agent via a medicinal product is considered serious and should be assessed under the category of medically important events in the absence of other seriousness criteria.

A Serious Adverse Event (SAE)/ Serious Adverse Reaction (SAR) is considered as serious when it fulfils at least one of the conditions for the definition of seriousness. An AE/ADR is considered as non-serious when it does not fulfil the conditions for the definition of seriousness.

Adverse Event (AE) / Adverse Drug Reaction (ADR) Intensity (Severity)

The intensity level of a serious or a non-serious AE or ADR is attributed according to the following definitions:

- Mild: does not interfere with routine activities; in case of laboratory tests when there is a mild abnormality.
- Moderate: interferes with the routine activities; in case of laboratory tests when there is a moderate abnormality.
- Severe: makes it impossible to perform routine activities; in case of laboratory tests when there is a significant abnormality.

Adverse Event (AE) / Adverse Drug Reaction (ADR) Expectedness

An AE/ADR is considered **unexpected** when the nature, severity, or outcome of the AE/ADR is not consistent with the information provided in the safety sections of the Investigator Brochure, which includes as the Reference Safety Information (RSI) the information of Summary of Product Characteristics (SmPC) of Nebivolol (Nebilet® 5 mg tablet) and Ramipril (Delix protect®, Cardace®, Triatec® or Tritace® 10 tablet).

The definition of ADR also covers medication errors and uses outside what is foreseen in the protocol, including overdose, misuse and abuse of the product.

Serious Unexpected Adverse Drug Reaction (SUSAR)

Any serious adverse event judged by the Investigator or the Sponsor as drug-related, i.e. Serious Adverse Reaction (SAR; see section 9.5.1.4) and unexpectedly qualifies as a Suspected Unexpected Serious Adverse Reaction (SUSAR). As a general rule for this clinical study, all SARs should be considered unexpected even if listed in the SmPCs of Nebivolol (Nebilet® 5 mg tablet) and/or Ramipril (Delix protect®, Cardace®, Triatec® or Tritace® 10 tablet). or even if a SAR of the same kind has already previously occurred during the clinical study.

Therefore, all SARs occurring in the clinical study NEB-RAM-01 will be processed in accordance with the applicable regulations for SUSARs. SUSARs are subject to expedited reporting, as specified in section 9.5.3.2, as having a “Reasonable Possibility” of relationship with the IMP.

In case of any SAR, subject enrolment and treatment administration will be put on-hold or stopped. The study will be resumed only after specific discussion and agreement with the IEC and the CA.

7.2.3. Definition of terms and generation/transformation of PK variables

Please refer to appendix A

7.2.4 Criteria for handling concentrations below LLOQ

Please refer to appendix A

7.2.5 Criteria for the calculation of λz and related parameters

Please refer to appendix A

7.2.6 Anomalous values and exclusion of data

Please refer to appendix A

7.3.Analysis populations

Safety population*:* All subjects receiving at least one administration of study treatment.

Pharmacokinetic (PK) population: All subjects who have evaluable and reliable concentration-time data for deriving the study primary PK parameters for both the Reference and Test formulations and who did not experience major protocol violations or events impacting the PK results.

7.4.On study and pre-study closure activities

7.4.1. Data monitoring

This study will be monitored in accordance with the ICH Guidelines for GCP.

Monitoring procedures require that 100% of data are source data verified, particularly focusing on informed consents, adherence to inclusion/exclusion criteria, drug accountability, documentation of SAEs and the proper recording of efficacy and safety measurements.

All monitoring activities will be described in detail in the study-specific monitoring plan.

7.4.2. Protocol Deviations and Data Review Meeting

Major or minor protocol deviations are defined as those deviations from the protocol likely to have an impact on the PK or safety of study treatments.

Before any analyses of the data, a data review meeting (DRM) will take place to identify protocol deviations and any other episode that could affect the evaluation and reliableness of concentration-time data. Such cases will be discussed, and on a case-by-case basis it will be determined whether to exclude the patients from the PK population. The final decisions on which patients to include or exclude from the PK populations will be finalized prior to database lock.

The following categories of protocol violation will be investigated in the DRM in order to identify subjects experiencing major or minor protocol violations:

- Informed consent not given

- Violation of inclusion/exclusion criteria (present at study start and/or developed during the study)
- No withdrawal from the study despite development of withdrawal criteria
- Violation of visit schedule (outside the window of acceptability defined in the study protocol)
- Violation from treatment
- Concomitant medication discordant with study protocol
- Factors interfering with drug uptake (e.g., vomiting after oral drug intake)
- Further protocol deviations not mentioned.

The minor and major protocol violations will be discussed during the review meeting and reported in the data review meeting report.

After the pre-analysis evaluation has been done, the database will be locked.

8. Determination of sample size

A sample size of 42 subjects (14 subjects per each of the sequences A, B and C) is considered adequate for testing the bioequivalence between each of the two NEB/RAM 5/10 mg FDC (Test formulation) and NEB 5mg + RAM 10 mg EC (Reference formulation) at 5% (one side) level of significance with a power higher than 80% based upon a Test versus Reference ratio varying between 0.95 and 1.05 and an intra-subject coefficient of variation of a maximum of 36.2%. [(1), (2)]

If a subject prematurely terminates his/her study participation for any reason not completing the three study sessions, he/she will be replaced with a new subject who will be randomised to the same sequence as per the replaced subject. The reason for replacement is based on the primary objective of the protocol i.e., to demonstrate the bioequivalence of Test vs Reference and study design (cross-over).

To account for approximately 22% of potential dropouts during the clinical phase and exclusions from the PK population, twelve extra subjects will be included into the study and a total number of 54 subjects will be randomized.

9. Randomization Methodology

The study will be performed according to an open design with treatment sequence allocated to each subject according to a randomization list; no blinding technique will be used.

The Global Biostatistic Department of Menarini Group will be responsible for generating the randomization list. The list should be kept at site. As soon as the subject's eligibility to be randomized is confirmed, the subject will be assigned to the lowest randomization number available in the list.

Each randomized subject will be allocated to one of the three treatment sequences.

Treatment sequences A, B and C are shown in table below:

	Study Session		
	1	2	3
Nebivolol/Ramipril 5/10 mg dose			
Sequence A	5/10 mg FDC	5/10 mg Extemporaneous Combination	5/10 mg Extemporaneous Combination
Sequence B	5/10 mg Extemporaneous Combination	5/10 mg Extemporaneous Combination	5/10 mg FDC
Sequence C	5/10 mg Extemporaneous Combination	5/10 mg FDC	5/10 mg Extemporaneous Combination

10.Stopping Rules and Blinding

10.1. Stopping Rules

Anytime, if a case of any serious adverse (drug) reaction (SAR) occurs, subject enrollment and treatment administration will be put on-hold or stopped. The study can be resumed only after the case has been discussed in a formal safety data review meeting with Sponsor’s Team and the Principal Investigators.

10.2.Blinding

This is an open-label study.

11.Statistical analysis and methods

11.1.Multiplicity adjustment

N/A

11.2.Descriptive statistics

All study variables will be presented by treatment/period/sequence and overall, by using the appropriate descriptive statistics according to the variable nature, unless otherwise specified:

- Continuous variables: number of non-missing observations, mean, standard deviation, standard error of the mean, minimum, median, maximum.
- Categorical variables: number of non-missing observations and column percentages (N, %).
- PK parameters: arithmetic mean (90% CI), Standard Deviation (SD), Coefficient of Variation (CV%), geometric mean (GM) (90% CI), geometric SD, geometric CV%, minimum, median and maximum.

Results will be presented by study populations, as appropriate.

When estimating the mean or median value for the concentration at a given time point (descriptive mean or median curve), a value of 0 will be assigned to below LLOQ (BLOQ) values. The mean/median value at a time with one or more BLOQ values will be reported (in tabular or graphical fashion) unless the mean/median value is below the LLOQ of the assay, in which case the value will be assigned BLOQ.

All the baseline characteristics will be summarized through descriptive statistics overall.

11.3.Data imputation

The missing values will not be imputed because an observed-cases approach will be applied.

It will be assumed that all pharmacokinetic parameters except t_{max} follow a lognormal distribution. In particular, C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ will be transformed with the natural logarithm for inferential analyses (ANOVA), in order to have normally distributed data.

11.4.Patient disposition and Baseline tables

The number of enrolled subjects will be used for the patient disposition summaries, which will be summarized by sequence and overall.

The following information will be provided:

1. Number (%) of patients who are screened.
2. Number (%) of patients who are screening failures.
3. Number (%) of patients who entered the Safety population.
4. Number (%) of patients who completed the study.
5. Number (%) of patients who discontinued study treatment.
6. Number (%) of patients who entered the PK population.

Demographic baseline characteristics will be based on both PK and safety populations and will provide descriptive statistics regarding age, BMI, height, weight, ethnicity, race and gender, summarized by sequence and overall. The same table will be also provided, for the PK population only, stratified by gender and overall.

11.5. Safety analysis

11.5.1 Safety assessment

The safety assessment will be based on the following measurements / evaluations performed to monitor subjects' safety along the study at specific time points according to the study flow-chart (section 5.3):

- Physical examination and vital signs.
- Safety laboratory tests.
- 12-lead ECG.

11.5.2 Adverse Events

The safety analysis will be run on the safety population and will encompass AEs, laboratory tests, physical examination, vital signs and 12-lead ECG data. It will be carried on by using descriptive statistics.

- AEs will be coded according to the last version of the MedDRA available at the start of the study.
- TEAEs will be presented by treatment/sequence and tabulated by causal relationship, intensity, seriousness and discontinuation of treatment. TEAEs will be also summarised by primary System Organ Class (SOC) and preferred term (PT). For each PT / SOC, number of TEAEs, number and percentage of subjects with TEAE will be overall listed and stratified by

intensity (mild, moderate and severe) and by their relationship to the study drugs (related/not related).

- SAEs will be analogously analysed as TEAEs.
- ADRs will be analogously analysed as TEAEs.
- AE/SAE not considered TEAE will be included in a separate listing
- Other Safety Variables: Descriptive statistics for absolute value and change of vital signs, 12-lead ECG, physical examination, laboratory tests will be presented by time point, sequence and overall. For these variables, the changes versus baseline (i.e. last assessment prior to dosing) will be presented by sequence and overall.

11.5.3. Physical Examination and Vital Signs

A summary table of Vital Signs parameters described in protocol section 9.4.3.3 will be provided in the TLFs. Descriptive statistics for absolute value and change versus baseline for vital signs (i.e., last assessment prior to dosing) will be presented by time point, treatment and overall.

Investigator judgement (clinically significant/not clinically significant) for vital signs will also be presented by sequence and time point.

Height (in cm), weight (in kg) and BMI calculation (is calculation will be performed in the eCRF) will be analyzed in the baseline table as discussed in section 11.2.

11.5.4. 12-ECG

The 12-lead ECGs will be performed after at least 10-minute rest in supine position, at the time points reported in the study flow chart (section 5.3). The Investigator or a designee will evaluate whether they are normal or abnormal and, if abnormal, whether they are clinically significant. Any occurrence of abnormalities in the cardiac conduction, depolarisation, repolarisation, arrhythmic events and other abnormalities will be evaluated.

The ECG report should be identified with subject details as well as the date and time of recording.

The dated and signed printed version of the ECG will be regarded as source data.

ECGs may be repeated for quality reasons and the results of the repeated ECG will be analysed. Unscheduled ECGs may be collected by the Investigator for safety reasons.

Clinically relevant abnormal findings detected after the signature of the ICF will be reported as AEs in the eCRF.

12- lead ECG parameters will be analyzed on the safety population by means of descriptive tables, similarly to Vital Signs, that will be provided in the TLF. ECG overall investigator judgement will be reported by descriptive statistics by sequence and time point.

11.5.5. Safety laboratory Test

Summary tables of clinical laboratory parameters described in protocol section 9.4.3.4 will be provided in the TLFs.

For biochemistry, haematology, and urinalysis the descriptive statistics and change versus baseline will be reported by analyte, sequence group and time point on the safety population.

For biochemistry, haematology, and urinalysis the Investigator assessment (Normal/ANCS/ACS) frequencies will be reported by analyte, sequence group and time point on the safety population.

For serology the descriptive statistics will be reported by sequence group and overall on the safety population.

For alcohol test, drugs of abuse test and cotinine Test the descriptive statistics will be reported by sequence and overall.

11.5.6. Other Safety variables

Descriptive statistics for absolute value and change of vital signs, 12-lead ECG, physical examination, laboratory tests will be presented by time point, sequence and overall. For these variables, the changes versus baseline (i.e., last assessment prior to dosing) will be presented by time point, sequence and overall.

12.Efficacy evaluations

12.1.Efficacy analysis

N/A

12.2.Subgroup analyses

N/A

12.3 Pharmacokinetic analysis

The PK analysis will be run on the PK population. All PK parameters will be summarised by descriptive statistics as detailed in section 11.2.

Individual concentration versus time profiles (linear and semi-log scales) for Nebivolol,

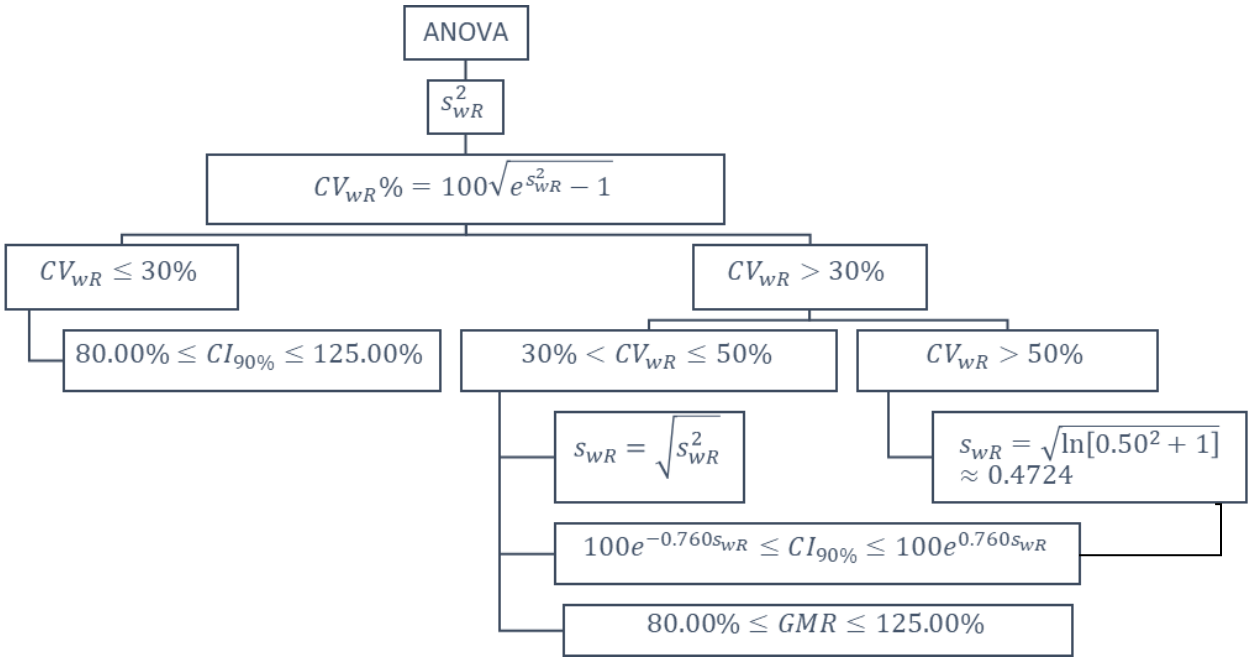
Ramipril and Ramiprilat will be graphically displayed by subject, treatment and period as appropriate. Mean PK concentrations by treatment and period will also be presented using both linear and semi-log scales. For PK parameters C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$, comparative boxplots will be provided for Nebivolol, Ramipril and Ramiprilat by treatment. Box-plots will also be produced for Ramiprilat Cmax and Ramiprilat and Nebivolol $AUC_{(0-72)}$ by treatment.

12.3.3 Analysis of Primary PK variables

Due to the replicate design adopted for the study, a dedicated approach will be performed to understand whether Ramipril is to be considered an HVD (high variable drug).

First, natural log transformed $AUC_{(0-t)}$ and C_{max} will be analysed using an ANOVA model including sequence, period treatment as fixed effects and subject within sequence as random effect.

After the statistical model has provided the results, the schematic approach below will be followed for Ramipril Cmax:



After calculating variance of Ramipril Cmax within Reference Formulation s^2_{wR} from ANOVA, the coefficient of variation within Reference Formulation CV_{wR} can be calculated with the below formula:

$$CV_{wR}\% = 100 * \sqrt{exp\ s^2_{wR} - 1}$$

If it is true that

$$CV_{wR} > 30\%$$

Then, as EMA's approach suggests, Ramipril will be considered highly variable. Then, s_{wR} will be estimated following this rule:

$$s_{wR} = \begin{cases} \sqrt{s_{wR}^2} & \textbf{if } 30.00\% < CV_{wR} \leq 50.00\% \\ \sqrt{\ln[0.50^2 + 1]} \approx 0.4724 & \textbf{if } CV_{wR} > 50\% \end{cases}$$

From the value of s_{wR} it is possible to calculate the limits of the acceptance confidence interval:

$$[L; U] = 100\ exp\ \{\mp k\ *\ s_{wR}\}$$

where k is the regulatory constant set to $k = 0.760$.

Below it can be seen some examples of how limits of acceptance confidence interval change according to different values of CV_{wR}.

CV_{wR}	Lower Limit L	Upper Limit U
30%	80.00%	125.00%
35%	77.23%	129.48%
40%	74.62%	134.02%
45%	72.15%	138.59%
≥ 50%	69.84%	143.19%

The possibility to widen the acceptance criteria explained above does not apply to the Ramipril $AUC_{(0-t)}$ where the acceptance range should remain at 80.00 – 125.00% regardless of variability.

As anticipated, natural log transformed C_{max} and $AUC_{(0-t)}$ of Nebivolol and Ramipril will be analysed using a mixed effect model including sequence, period and formulation as fixed effects and subject within sequence as random effect.

Estimates of the mean difference (Test formulation – Reference formulation) and corresponding 90% CIs will be obtained from the model for each analyte.

The mean differences and 90% CIs for the differences will be exponentiated to provide estimates of the ratio of geometric means (Test formulation/Reference formulation) and corresponding 90% CIs.

Bioequivalence will be concluded if the 90% CI for the ratio of geometric means for both C_{max} and $AUC_{(0-t)}$ falls wholly within the acceptance range for both Nebivolol and Ramipril. The acceptance range will be $80.00\% - 125.00\%$ for all tests except for Ramipril's C_{max} if it will be considered highly variable. In this case, limits of acceptance confidence interval will be calculated as explained above and the bioequivalence will be considered verified if the 90% CI for the GMR of Ramipril's C_{max} falls wholly within the acceptance range and the punctual value of GMR falls within the acceptance range of $80.00\% - 125.00\%$.

Formally, the following Bioequivalence tests will be performed to assess the primary objective:

1. Ramipril of Test formulation versus Ramipril of Reference formulation in term of $AUC_{(0-t)}$
2. Ramipril of Test formulation versus Ramipril of Reference formulation in term of C_{max}
3. Nebivolol of Test formulation versus Nebivolol of Reference formulation in term of $AUC_{(0-t)}$
4. Nebivolol of Test formulation versus Nebivolol of Reference formulation in terms of C_{max}.

For the $AUC_{(0-t)}$ and for Nebivolol's C_{max}, following the ±20% rule, the bioequivalence will be concluded if the mean bioavailability of the test formulation, μ_T, is within ±20% of the mean of the reference formulation, μ_R, i.e., in terms of a ratio of means, if $0.8 < \mu_T / \mu_R < 1.25$. The same limits will be applied if Ramipril won't be considered highly variable, otherwise the limits used for the bioequivalence will be those calculated as explained above. Using the terminology of statistical hypothesis testing, this is accomplished by testing, for each of the eight tests, the hypothesis:

$$H_0: \frac{\mu_T}{\mu_R} \leq L \; or \; \frac{\mu_T}{\mu_R} \geq U \;\; vs \;\; H_1: L \leq \frac{\mu_T}{\mu_R} \leq U$$

Where $L = 0.8$ and $U = 1.25$ for $AUC_{(0-t)}$ of both Nebivolol and Ramipril and Nebivolol's C_{max}, while for C_{max} of Ramipril they will be evaluated.

The SAS syntax used for ANOVA procedure for each of the four tests will be:

PROC MIXED DATA=dataset;

CLASS formulation subject period sequence;

MODEL log(pk variable)= sequence period formulation;

RANDOM subject(sequence);

ESTIMATE "Test vs Reference" formulation -1 1 / CL ALPHA=0.10;

RUN;

The SAS syntax used to estimate s^2_{wR} will be, considering the dataset with only Reference formulation, the following one:

PROC GLM DATA = dataset (with only data from Reference formulation)

CLASS subject period sequence;

MODEL log (PK variable) = sequence subject(sequence) period;

RUN;

In this way it is possible to obtain in the SAS output the Mean Square Error, so that it can be calculated $CV_{wR}\%$ as explained above.

To reflect the multigroup nature of the study, a sensitivity analysis of the above ANOVA model will be performed after the main bioequivalence analysis including the group effect to the other pre-existing covariates. The term group is based on the day in which the subject was dosed, i.e., all subjects who received their first intake of a study drug on the same day will be assigned to the same group. This sensitivity analysis is useful to understand if the interaction between group and treatment is significant or not. Note that if the groups are not well balanced and not separated in time, statistical analysis using formulation*group term may not be conclusive.

The SAS syntax used for ANOVA procedure including the group effect will be:

PROC MIXED DATA=dataset;

CLASS subject sequence formulation period group;

*MODEL log(pk variable)=group sequence formulation sequence*group period(group) formulation*group;*

LSMEANS formulation;

*RANDOM subject(sequence*group)*

ESTIMATE "Test vs Reference" formulation -1 1/ CL ALPHA= 0.1;

RUN;

If the formulation*group interaction term is found to be statistically significant and if the size of the groups is considered adequate for inference purposes (i.e., the groups are balanced and greatly separated in time), the primary ANOVA model for assessing the bioequivalence of each analyte will be run within each group.

12.3.4 Analysis of secondary PK variables

- The following secondary PK variables for the analytes Nebivolol and Ramipril will be compared when administered as FDC tablet vs. EC:
  - $AUC_{(0-\infty)}$ will be analysed similarly to $AUC_{(0-t)}$.
  - Other PK parameters will be summarized descriptively.

12.3.5 Analysis of exploratory PK variables

Exploratory PK parameters will be summarised descriptively. The bioequivalence testing may also be conducted for Ramiprilat in case of inconclusive results for the BE based on Ramipril.

13. Tables, listings and figures

13.1. Statistical Analysis Report

The TLF (Tables, Listings and Figures) will follow the list of tables, plots, and listings agreed with the other teams involved in the study conduction.

13.2. Index of TLFs

13.2.1 Tables

Subject Disposition

- Table 1.1: Overall subject disposition by sequence
- Table 1.2: Presence of subjects at study visits by sequence – Analysis Population: Safety

Demographics and baseline characteristics

- Table 2.1: Demographics and baseline characteristics by sequence – Analysis Population: Safety
- Table 2.2: Demographics and baseline characteristics by sequence – Analysis Population: PK
- Table 2.3: Demographics characteristics by gender – Analysis Population: PK
- Table 2.4: Pregnancy test by time point and sequence - Analysis Population: Safety

Pharmacokinetic analysis (PK population)

- Table 1.1: Bioequivalence analysis for $AUC_{(0-t)}$, C_{max} and $AUC_{(0-\infty)}$ of Nebivolol – Analysis Population: PK population
- Table 1.2: Bioequivalence analysis for $AUC_{(0-t)}$, C_{max} and $AUC_{(0-\infty)}$ of Ramipril – Analysis Population: PK population
- Table 1.3: Descriptive statistics of Nebivolol PK parameters by treatment and period – Analysis Population: PK population
- Table 1.4: Descriptive statistics of Ramipril PK parameters by treatment and period – Analysis Population: PK population
- Table 1.5: Descriptive statistics of Ramiprilat PK parameters by treatment and period – Analysis Population: PK population
- Table 1.6: Descriptive statistics of Plasma Concentrations of Nebivolol (after FDC intake) by sequence and time point – Analysis Population: PK population
- Table 1.7: Descriptive statistics of Plasma Concentrations of Nebivolol (after extempora neous combination intake) by sequence and time point – Analysis Population: PK population
- Table 1.8: Descriptive statistics of Plasma Concentrations of Ramipril (after FDC intake) by sequence and time point – Analysis Population: PK population

- Table 1.9: Descriptive statistics of Plasma Concentrations of Ramipril (after extemporaneous combination intake) by sequence and time point – Analysis Population: PK population
- Table 1.10: Descriptive statistics of Plasma Concentrations of Ramiprilat (after FDC intake) by sequence and time point – Analysis Population: PK population
- Table 1.11: Descriptive statistics of Plasma Concentrations of Ramiprilat (after extemporaneous combination intake) by sequence and time point – Analysis Population: PK population
- Table 1.12: Descriptive statistics of PK parameters of Nebivolol (after FDC intake) by sequence – Analysis Population: PK population
- Table 1.13: Descriptive statistics of PK parameters of Nebivolol (after extemporaneous combination intake) by sequence – Analysis Population: PK population
- Table 1.14: Descriptive statistics of PK parameters of Ramipril (after FDC intake) by sequence – Analysis Population: PK population
- Table 1.15: Descriptive statistics of PK parameters of Ramipril (after extemporaneous combination intake) by sequence – Analysis Population: PK population
- Table 1.16: Descriptive statistics of PK parameters of Ramiprilat (after FDC intake) by sequence – Analysis Population: PK population
- Table 1.17: Descriptive statistics of PK parameters of Ramiprilat (after extemporaneous combination intake) by sequence – Analysis Population: PK population
- Table 1.18 Bioequivalence analysis for $AUC_{(0-t)}$, C_{max} and $AUC_{(0-\infty)}$ of Nebivolol including group effect (sensitivity analysis) – Analysis Population: PK population
- Table 1.19: Bioequivalence analysis for $AUC_{(0-t)}$, C_{max} and $AUC_{(0-\infty)}$ of Ramipril including group effect (sensitivity analysis) – Analysis Population: PK population

Safety analysis (safety population)

- Table 2.1.1.1: Overview AEs by Sequence – Analysis Population: Safety
- Table 2.1.1.1: Overview AEs by Treatment – Analysis Population: Safety
- Table 2.1.1: Overview TEAEs by Sequence – Analysis Population: Safety
- Table 2.1.2: Overview ADRs by Sequence – Analysis Population: Safety
- Table 2.2.1: Overview TEAEs by Treatment – Analysis Population: Safety
- Table 2.2.2: Overview ADRs by Treatment – Analysis Population: Safety
- Table 2.3.1: TEAEs by MedRa, SOC and PT by Sequence – Analysis Population: Safety
- Table 2.3.2: ADRs by MedRa, SOC and PT by Sequence – Analysis Population: Safety
- Table 2.3.3: TEAEs by MedRa, SOC and PT by Intensity and Sequence - Analysis Population: Safety
- Table 2.3.4: TEAEs by MedRa, SOC and PT by Causality and Sequence – Analysis Population: Safety
- Table 2.3.5: ADRs by MedRa, SOC and PT by Intensity and Sequence – Analysis Population: Safety
- Table 2.3.6: ADRs by MedRa, SOC and PT by Causality and Sequence – Analysis Population: Safety
- Table 2.4.1: TEAEs by MedRa, SOC and PT by Treatment – Analysis Population: Safety
- Table 2.4.2: ADRs by MedRa, SOC and PT by Treatment – Analysis Population: Safety

- Table 2.4.3: TEAEs by MedRa, SOC and PT by Intensity and Treatment – Analysis Population: Safety
- Table 2.4.4: TEAEs by MedRa, SOC and PT by Causality and Treatment – Analysis Population: Safety
- Table 2.4.5: ADRs by MedRa, SOC and PT by Intensity and Treatment – Analysis Population: Safety
- Table 2.4.6: ADRs by MedRa, SOC and PT by Causality and Treatment – Analysis Population: Safety
- Table 2.5: Haematology by sequence and Visit and their change versus baseline – Analysis Population: Safety
- Table 2.6: Biochemistry by sequence and Visit and their change versus baseline – Analysis Population: Safety
- Table 2.7: Urinalysis by sequence and Visit and their change versus baseline – Analysis Population: Safety
- Table 2.8: Vital signs by sequence and Time Point and their change versus baseline – Analysis Population: Safety
- Table 2.9: ECG by sequence and Time Point and their change versus baseline – Analysis Population: Safety
- Table 2.10: Haematology Investigator Judgment by Sequence and visit – Analysis Population: Safety
- Table 2.11: Biochemistry Investigator Judgment by Sequence and visit – Analysis Population: Safety
- Table 2.12: Urinalysis Investigator Judgment by Sequence and visit – Analysis Population: Safety
- Table 2.13: ECG Investigator Judgment by Sequence and time point – Analysis Population: Safety
- Table 2.14: Vital signs Investigator Judgment by Sequence and time point – Analysis Population: Safety
- Table 2.15: Serology by sequence (HIV-1, HIV-2, hepatitis B, hepatitis C and SARS-CoV-2) – Analysis Population: Safety
- Table 2.16: Toxicology: Alcohol breath test, drugs abuse and Cotinine Test by sequence – Analysis Population: Safety

13.2.2. Listings

The following subject data listings will always contain the following key variables (KV): subject id, analysis population [safety, pk], period, age at screening, gender.

L1. Study visits and course of study (only safety population)
KV, period, sequence, start date, end date, course of study.

L2. Demographic data and baseline subject information
KV, date of informed consent, height, weight, BMI, race, ethnicity, childbearing potential, CYP2D6 test.

L3. Medical history
KV, disease, system organ class, start date, end date, ongoing.

L4. Plasma Concentration
KV, period, planned time-point, scheduled time, actual time, concentration, LLOQ.

L5. Plasma PK parameters
KV, period, pk parameters

L6. Vital Signs
KV, period, date and time, parameters, result, unit, range, investigator's judgment.

L7. 12-lead ECG
KV, period, date and time, result, investigator judgment.

L8. Drugs of abuse, Alcohol test and Cotinine Test
KV, period, date and time, test, result.

L9. Serology
KV, period, date and time, test, result

L10. Laboratory safety test: Haematology/Biochemistry
KV, period, date and time, test, result, unit, reference range, investigator's judgment.

L11. Laboratory safety test: Urinalysis
KV, period, date and time, test, value, overall investigator's judgment.

L12. Pregnancy Test
KV, period, date and time, test, result [Positive/Negative]

L13. Concomitant medication
KV, period number, drug, dose, unit, frequency, route, indication, start medication date, end medication date/ongoing, ATC coding.

L14. Adverse events
KV, period, treatment, AE Num., AE verbatim, onset date and time, end date and time, ongoing, pattern, intensity, causality (by Investigator and by Sponsor), action taken, report type, outcome, SAE [yes/no] (by Investigator and by Sponsor), treatment emergent flag.

L15. Adverse events coding
KV, period, treatment, AE Num., system organ class, preferred term, low level term, Date-time of first exposure in PK session 1, Date-time of first exposure in PK session 2, Date-time of first exposure in PK session 3.

L16. Drug administration
KV, period, treatment administered, date and time, kit number.

13.2.3. Figures – to be provided by CROSS Research S.A

- Figure 1.1: Individual concentration versus time profiles by subject and treatment for Nebivolol - Linear Scale
- Figure 1.2: Individual concentration versus time profiles by subject and treatment for Nebivolol – Semi Log Scale
- Figure 1.3: Mean (SD) PK profiles for Nebivolol by treatment – Linear Scale
- Figure 1.4: Mean (SD) PK profiles for Nebivolol by treatment – Semi Log Scale
- Figure 2.1: Individual concentration versus time profiles by subject and treatment for Ramipril - Linear Scale
- Figure 2.2: Individual concentration versus time profiles by subject and treatment for Ramipril – Semi Log Scale
- Figure 2.3: Mean (SD) PK profile for Ramipril by treatment – Linear Scale
- Figure 2.4: Mean (SD) PK profile for Ramipril by treatment – Semi Log Scale
- Figure 3.1: Individual concentration versus time profiles by subject and treatment for Ramiprilat - Linear Scale
- Figure 3.2: Individual concentration versus time profiles by subject and treatment for Ramiprilat – Semi Log Scale
- Figure 3.3: Mean (SD) PK profile for Ramiprilat by treatment – Linear Scale
- Figure 3.4: Mean (SD) PK profile for Ramiprilat by treatment – Semi Log Scale
- Figure 4.1: Comparative boxplots of $AUC_{(0\text{-}t)}$, $AUC_{(0\text{-}\infty)}$, $AUC_{(0\text{-}72h)}$ and C_{max} of Nebivolol by treatment
- Figure 4.2: Comparative boxplots of $AUC_{(0\text{-}t)}$, $AUC_{(0\text{-}\infty)}$ and C_{max} of Ramipril by treatment
- Figure 4.3: Comparative boxplots of $AUC_{(0\text{-}72h)}$ and C_{max} of Ramiprilat by treatment

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Appendix A

A1. Introduction

The purpose of this appendix is to provide further details about the analysis methods for the generation of the PK parameters that will be provided by CROSS Research S.A.

A2. CROSS Standard Operating Procedures (SOPs) to be followed

Code	Title
SOP-11-01-09	Pharmacokinetic analysis
Pol-0009	Pharmacokinetic conventions

These SOPs could not be strictly followed in case the sponsor requires his standard.

A3. Definition of terms and generation/transformation of PK variables

Pharmacokinetic parameters will be presented in data listings and summarized in tables by analyte, treatment, using descriptive statistics (n, arithmetic mean (90% CI), SD, CV %, geometric mean (90% CI), geometric mean CV%, geometric SD, minimum, median, and maximum).

The following PK parameters will be determined by non-compartmental analysis (NCA) of the individual plasma concentration-time profiles of Nebivolol, Ramipril and Ramiprilat for the Test and Reference formulations using Phoenix™ WinNonlin® software, version 8.3.5:

C_{max}	Maximum plasma concentration
t_{max}	Time to C_{max}
C_{last}	Last quantifiable plasma concentration observed
t_{last}	Time corresponding to C_{last}
λ_z	Apparent terminal elimination rate constant, estimated by log-linear regression analysis on plasma concentrations visually assessed to be on the terminal log-linear phase.
$t_{1/2}$	Plasma terminal half-life, calculated according to the following equation: $t_{1/2} = \frac{0.693}{\lambda_z}$
$AUC_{(0\text{-}t)}$	Area under the plasma concentration-time curve from time zero (predose) to the time of the last quantifiable concentration, calculated by means of the linear-log trapezoidal method
$AUC_{(0-\infty)}$	Area under the plasma concentration-time curve from time zero to infinity, calculated according to the following equation (for nebivolol and ramipril only): $AUC_{(0\text{-}\infty)} = AUC_{(0-t)} + \frac{C_{last}}{\lambda_z}$
$AUC_{(0\text{-}72)}$	Area under the plasma concentration-time curve from time zero (predose) to 72h post-dose (for nebivolol and ramiprilat only)
$\%AUC_{ex}$	The percentage of AUC ($_{0-\infty}$) obtained by extrapolation ($\%AUC_{ex}$) will be calculated as follows (for nebivolol and ramipril only): $\%AUC_{ex} = \frac{AUC_{(0\text{-}\infty)} - AUC_{(0\text{-}t)}}{AUC_{(0\text{-}\infty)}} \times 100$

Other PK parameters can be calculated, if considered appropriate and justified at the time of the PK analysis.

Actual PK sampling times will be used in the derivation of non-compartmental PK parameters. Nominal sampling times may be used as a replacement for unknown or missing actual times.

All digits will be used for PK parameter calculations and statistical analyses. For PK data presented in summary tables and listings, values will be presented by rounding off to two decimal digits, except for the following situations (this applies to individual data and descriptive statistics):

• λ_z and R_{sq} adjusted data: rounded off to four decimal digits.

• PK parameters related to time such as t_{max}, λ_z Lower, and λ_z Upper must be reported with the same precision as the actual sampling time (i.e., rounded off to 3 decimal digits).

• Concentration versus time data as well as C_{max}: reported as they appear in the corresponding dataset.

Descriptive statistics for PK data will be presented with the same precision as the PK parameters.

A4. Criteria for handling concentrations below LLOQ

- Concentration values below the Lower Limit of Quantification of the bioanalytical method (LLOQ) will be set to zero;
- Concentrations reported as >LLOQ at time zero, when the subject has not previously been dosed, will be set to their real value.

A5. Criteria for the calculation of λz and related parameters

- The best-fit method in Phoenix WinNonlin will be used to estimate λ_z from at least 3 concentration data points excluding the Cmax. Time range method (manual selection) might be used on a case-by-case basis if the best-fit method does not provide the most appropriate estimate of λ_z.
- R_{sq} adjusted, i.e., the goodness of fit statistic for the terminal elimination phase adjusted for the number of points used in the estimation of λ, must be ≥ 0.8.
- If the λ_z cannot be measured (e.g.: less than 3 non-zero concentrations in the terminal elimination phase or R_{sq} adjusted < 0.8), the PK parameters derived from λ_z ($AUC_{(0-\infty)}$, % AUC_{extrap} and $t_{1/2}$) will not be calculated. If applicable, the time point where ln-linear λ_z calculation begins ($\lambda_{z\ Lower}$) and the actual sampling time of the last measurable concentration used to estimate the λ_z ($\lambda_{z\ Upper}$), as well as the R_{sq} adjusted for the ln-linear regression for the calculation of the elimination rate constant will be reported.
- If Span (i.e., Ratio between the sampling interval of the measurements used for the λz and the terminal half-life) is < 2, λ_z-related parameters will be presented and included in the descriptives statistics, but flagged in listings;
- The % AUC_{extrap} should not exceed 20% for each individual profile. If the %AUC_{extrap} is more than 20% (i.e. $AUC_{(0-t)}$ covers less than 80% of $AUC_{(0-\infty)}$), the individual result should be flagged but included in the descriptive statistics.

A6. Anomalous values and exclusion of data

Individual concentrations deemed to be anomalous will not be excluded from the PK analysis and mean profiles; anyway, such anomalous values will be identified in the relevant tables of study report. Anomalous values are those that are inconsistent with known or expected PK behaviour of the drug and are not defined in a statistical outlier sense. Exclusion of data can be accepted only under the following circumstances:

1. Drop out/withdrawn subjects.
2. A subject with lack of any measurable concentrations or only very low plasma concentrations after Reference administration. A subject is considered to have very low plasma concentrations if its AUC is less than 5% of Reference geometric mean (GM) AUC (which should be calculated without inclusion of data from the outlying subject).
3. Subjects with non-zero pre-dose concentrations, i.e., > 5% of C_{max}. All data from such subjects will be excluded from bioequivalence calculation. The above can be due to subjects' non-compliance and/or to an insufficient wash-out period.
4. A subject may be excluded from the PK population if there are any important protocol deviations or adverse events (AEs) that may impact PK, for example: an AE of vomiting or diarrhoea, use of disallowed concomitant medications within the specified exclusion periods, incorrect or missed doses, missed samples, incorrect handling of the PK samples for analysis, dose and sample times not recorded or recorded incorrectly, or any other issue that may impact the integrity of the PK data.

Clear justification will be provided in the report for exclusion of any data.