TRIAL STATISTICAL ANALYSIS PLAN

c21690485-01

BI Trial No.:	0352-2100
Title:	The effect of potent inhibitors of drug transporters (verapamil, rifampin, cimetidine, probenecid) on pharmacokinetics of a transporter probe drug cocktail consisting of digoxin, furosemide, metformin and rosuvastatin (an open-label, randomised, crossover trial in three parts)
Investigational Product:	Digoxin, furosemide, metformin, rosuvastatin, verapamil, probenecid, cimetidine, rifampin
Responsible trial statisticians:	Phone: Fax: Phone: Fax:
Date of statistical analysis plan:	07 JUN 2018 SIGNED
Version:	Final

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2. LIST OF ABBREVIATIONS

Term	Definition / description
ADS	Analysis Dataset
AE	Adverse Event
AESI	Adverse Event of Special Interest
ANOVA	Analysis of Variance
AUC_{0-tz}	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 to the last quantifiable data point
$AUC_{0-\infty}$	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 extrapolated to infinity
BI	Boehringer Ingelheim
BWC	Bioavailability/Bioequivalence, Within-Subject Design, Time-Controlled
CI	Confidence Interval
$CL_{R,t1-t2}$	Renal clearance of the analyte in plasma from the time point t1 to t2
C_{max}	Maximum measured concentration of the analyte in plasma
CRF	Case Report Form
CTP	Clinical Trial Protocol
CTR	Clinical Trial Report
CV	Arithmetic Coefficient of Variation
DBLM	Database Lock Meeting
ECG	Electrocardiogram
fe_{t1-t2}	Fraction of given drug excreted unchanged in urine from time point t1 to t2
gCV	Geometric Coefficient of Variation
gMean	Geometric Mean
ICH	International Conference On Harmonisation
LLT	Lower Level Term
Max	Maximum
MedDRA	Medical Dictionary For Regulatory Activities
Min	Minimum
O*C	Oracle Clinical
PK	Pharmacokinetic(s)
PKS	PK parameter analysis set
PT	Preferred Term

Term	Definition / description
PV	Protocol Violation
qd	Quaque die (daily)
qid	Quater in die (4 times a day)
R	Reference Treatment
RAGe	Report Appendix Generator system
REP	Residual Effect Period
RPM	Report Planning Meeting
RS	Randomised set
SAS®	Statistical Analysis System
SD	Standard Deviation
SOC	System Organ Class
T	Test treatment
t_{max}	Time from dosing to maximum measured concentration of the analyte in plasma
TS	Treated Set
TSAP	Trial Statistical Analysis Plan
WHO-DD	World Health Organization Drug Dictionary
XPKISTAT	Library of SAS® Macros for PK analysis

3. INTRODUCTION

As per ICH E9 (1), the purpose of this document is to provide a more technical and detailed elaboration of the principal features of the analysis described in the protocol, and to include detailed procedures for executing the statistical analysis of the primary and secondary variables and other data.

This trial statistical analysis plan (TSAP) assumes familiarity with the Clinical Trial Protocol (CTP), including Protocol Amendments. In particular, the TSAP is based on the planned analysis specification as written in CTP Section 7 "Statistical Methods and Determination of Sample Size". Therefore, TSAP readers may consult the CTP for more background information on the study, e.g., on study objectives, study design and population, treatments, definition of measurements and variables, planning of sample size, randomisation.

Study data will be stored in a trial database within the Oracle Clinical™ (O*C) system.

Pharmacokinetic (PK) parameters will be calculated using Phoenix WinNonlin™ software (version 6.3 or higher, Certara USA Inc., Princeton, NJ, USA).

The statistical analyses will be performed within the validated working environment CARE, including SAS™ (current Version 9.4, by SAS Institute Inc., Cary, NC, USA), and a number of SAS™-based tools (e.g., macros for the analyses of AE data or laboratory data; Report Appendix Generator system (RAGe) for compilation/formatting of the CTR appendices).

4. CHANGES IN THE PLANNED ANALYSIS OF THE STUDY

All analyses as planned in the CTP will be performed and are described in more detail in this TSAP. The following change compared to the protocol will be made:

No 'Randomised set' (RS) will be defined in the TSAP as data of subjects randomised but discontinued before first administration of trial medication will not be entered in the case report form. A correct display of the RS would not be possible.

The follow-up and post treatment phase defined in the CTP will be combined to one post-treatment phase ("Post-trt") for Listings and Tables of Adverse events.

5. ENDPOINTS

5.1 PRIMARY ENDPOINTS

Section 5.5.1.1 of the CTP:

The following primary endpoints will be determined for digoxin, furosemide, metformin, and rosuvastatin (at cocktail doses):

- *AUC_{0-tz} (area under the concentration-time curve of the analyte in plasma over the time interval from 0 to the last quantifiable data point)*
- *C_{max} (maximum measured concentration of the analyte in plasma)*

5.2 SECONDARY ENDPOINTS

5.2.1 Key secondary endpoints

This section is not applicable as no key secondary endpoints have been defined in the CTP.

5.2.2 Secondary endpoints

Section 5.5.1.2 of the CTP:

The following secondary endpoint will be evaluated for digoxin, furosemide, metformin, and rosuvastatin (at cocktail doses):

- *$AUC_{0-\infty}$ (area under the concentration-time curve of the analyte in plasma over the time interval from 0 extrapolated to infinity)*

For digoxin, $AUC_{0-\infty}$ will be evaluated only if determination with sufficient precision is possible.

5.3 FURTHER ENDPOINTS

Pharmacokinetic (PK):

Pharmacokinetic parameters are further parameters of interest. For more details, see CTP Section 5.5.1.3.

For secondary PK analyses the following endpoints for digoxin, furosemide, metformin, and rosuvastatin (at cocktail doses) are of particular relevance:

- fe_{t1-t2} (fraction of given drug excreted unchanged in urine from time point t_1 to t_2)
- $CL_{R,\,t1-t2}$ (renal clearance of the analyte in plasma from the time point t_1 to t_2)

For further PK analyses, the following endpoints for furosemide and metformin given at therapeutic doses are of particular relevance:

- AUC_{0-tz} (area under the concentration-time curve of the analyte in plasma over the time interval from 0 to the last quantifiable data point)
- C_{max} (maximum measured concentration of the analyte in plasma)
- $AUC_{0-\infty}$ (area under the concentration-time curve of the analyte in plasma over the time interval from 0 extrapolated to infinity)

Safety:

Further criteria of interest:

- AEs (including clinically relevant findings from the physical examination)
- Safety laboratory tests
- 12-lead ECG
- Vital signs (blood pressure, pulse rate)

For detailed information (formula) please refer to Section 7.8.

5.4 OTHER VARIABLES

Section 5.2.5.2 of the CTP: *At screening, the medical examination will include demographics including height and body weight, smoking and alcohol history, relevant medical history and concomitant therapy* […].

Age [years] will be determined as the difference between year of informed consent and year of birth.

BMI will be calculated as weight [kg] / (0.01 * height [cm])^2.

6. GENERAL ANALYSIS DEFINITIONS

6.1 TREATMENTS

For basic study information on investigational products, assignment of treatment sequences, and selection of doses, please see CTP, Sections 3 and 4.

The study will be performed as a randomised, open-label, crossover trial in three parts.

In total, it was planned to assign 47 healthy male subjects to the three trial parts; 15 healthy male subjects will enter trial part 1, 16 healthy males will enter trial part 2 and 16 healthy males will enter trial part 3.

For details of dosage and formulation, see Tables 6.1: 1, 6.1: 2 and 6.1: 3 below.

Table 6.1: 1 Treatments and labels used in the analysis (Trial Part 1)

Treatment		Treatment long label*	Short label
A	R1	Digoxin 0.25mg + Furosemide 1mg + Metformin 10mg + Rosuvastatin 10mg	Cocktail (R1)
B	T1	Verapamil 120mg + Digoxin 0.25mg + Furosemide 1mg + Metformin 10mg + Rosuvastatin 10mg	R1+Vera (T1)
C	T2	Rifampin 600mg + Digoxin 0.25mg + Furosemide 1mg + Metformin 10mg + Rosuvastatin 10mg	R1+Rifa (T2)

* Please note:
The above listed treatment long labels do not reflect the actual treatment regimens from Oracle Clinical. These treatment long labels are a little bit more comprehensive.

Table 6.1: 2 Treatments and labels used in the analysis (Trial Part 2)

Treatment		Treatment long label*	Short label
A	R1	Digoxin 0.25mg + Furosemide 1mg + Metformin 10mg + Rosuvastatin 10mg	Cocktail (R1)
D	R2	Metformin, oral solution, 500mg	Met (R2)
E	T3	Day 1: Cimetidine 400mg tablet, qid + Digoxin 0.25mg + Furosemide 1mg + Metformin 10mg + Rosuvastatin 10mg Day 2: Cimetidine 400 mg bid	R1+Cime (T3)
F	T5	Day 1: Cimetidine, 400 mg tablet, qid + Metformin, oral solution, 500mg Day 2: Cimetidine 400 mg bid	R2+Cime (T5)

* Please note:
The above listed treatment long labels do not reflect the actual treatment regimens from Oracle Clinical. These treatment long labels are a little bit more comprehensive.

Table 6.1: 3 Treatments and labels used in the analysis (Trial Part 3)

Treatment		Treatment long label*	Short label
A	R1	Digoxin 0.25mg + Furosemide 1mg + Metformin 10mg + Rosuvastatin 10mg	Cocktail (R1)
K	R3	Furosemide, oral solution, 40mg, qd	Furo (R3)
L	T4	Day -1: Probenecid 2*500mg tablet qd Day 1: Probenecid 2*500mg tablet, qd + Digoxin 0.25mg + Furosemide 1mg + Metformin 10mg + Rosuvastatin 10mg	R1+Prob (T4)
M	T6	Day -1: Probenecid 2*500mg tablet qd Day 1: Probenecid,2*500 mg tablet, qd + Furosemide, oral solution, 40mg, qd	R3+Prob (T6)

* Please note:
The above listed treatment long labels do not reflect the actual treatment regimens from Oracle Clinical. These treatment long labels are a little bit more comprehensive.

The following separate study phases will be defined for the analyses of AEs:

- **Screening** (ranging from 0:00 h on day of informed consent until first administration time of study drug)
- **On treatment**
  (separately for each treatment, including residual effect period (REP); i.e. ranging from administration time of study drug until 7 days thereafter)
- **Post-treatment** (ranging from end of on treatment phase until next drug administration or 0:00 h on the day after trial termination date (labelled "**Post-trt**"))

Displays of AEs will be presented separately for the treatments described in Table 6.1: 1, Table 6.1: 2 and Table 6.1: 3 above.

Two types of AE displays will be provided in the report:

A) Section 15.3 (separately for all trial parts) and Appendix 16.1.13.1.8 (for ClinicalTrials.gov (separately for all trial parts) and EudraCT (all trial parts combined)) of the CTR displays:

In these displays, the on treatment phase will be analysed (labelled with the name of the study treatment (short label)). Screening and Post-treatment will not be included in this analysis. The following total will be provided in addition:

- a total over all on treatment phases included in this analysis ("**Total on treatment**") (Section 15.3 only)

B) Section 15.4 and Appendix 16.1.13.1.8 (except for ClinicalTrials.gov and EudraCT) of the CTR displays:

- Screening
- On treatment (labelled with the name of the study treatment (short label))
- Post-treatment

In Section 16.1.13.1.8 AE tables (separately for all trial parts), the following totals will be provided in addition:

- a total over all study phases ("**Total**")

Tables of vital signs and laboratory values will present results by the above mentioned on treatment phase.

For detailed information on the handling of the treatments in the O*C views, refer to Technical TSAP ADS (analysis data set) plan.

The following Table 6.1: 4 gives an overview of the planned analyses per trial part with the respective endpoints used to assess the pairwise comparisons for the investigated analyses.

Table 6.1: 4 Overview of intra-individual comparisons

Analysis	**Trial part**	**Analyte**	**Endpoints**	**Test treatment (T)**	**Reference treatment (R)**
Primary + sensitivity	Trial part 1	Digoxin Furosemide Metformin Rosuvastatin	AUC_{0-tz} C_{max} $AUC_{0-\infty}$	T1 T2	R1 R1
	Trial part 2	Digoxin Furosemide Metformin Rosuvastatin	AUC_{0-tz} C_{max} $AUC_{0-\infty}$	T3	R1
	Trial part 3	Digoxin Furosemide Metformin Rosuvastatin	AUC_{0-tz} C_{max} $AUC_{0-\infty}$	T4	R1
Secondary	Trial part 1	Digoxin Furosemide Metformin Rosuvastatin	$CL_{R,t1-t2}$ fe_{t1-t2}	T1 T2	R1 R1
	Trial part 2	Digoxin Furosemide Metformin Rosuvastatin	$CL_{R,t1-t2}$ fe_{t1-t2}	T3	R1
	Trial part 3	Digoxin Furosemide Metformin Rosuvastatin	$CL_{R,t1-t2}$ fe_{t1-t2}	T4	R1

Table 6.1: 4 Overview of intra-individual comparisons (cont.)

Analysis	Trial part	Analyte	Endpoints	Test treatment (T)	Reference treatment (R)
Further	Trial part 2	Metformin (500mg)	AUC_{0-tz} C_{max} $AUC_{0-\infty}$ $CL_{R,t1-t2}$ fe_{t1-t2}	T5	R2
	Trial part 3	Furosemide (40mg)	AUC_{0-tz} C_{max} $AUC_{0-\infty}$ $CL_{R,t1-t2}$ fe_{t1-t2}	T6	R3

Please note, that $AUC_{0-\infty}$ will be evaluated for digoxin if determination with sufficient precision is possible.

6.2 IMPORTANT PROTOCOL VIOLATIONS

Data discrepancies and deviations from the CTP will be identified for all treated subjects. Listings of protocol deviations and of unresolved discrepancies will be provided to be discussed at the combined report planning and database lock meeting (RPM/DBLM), e.g. deviations in drug administration, in blood sampling, etc. At this meeting, it will be decided whether the discrepant data can be used as they are or whether the data have to be corrected in the clinical database.

Each protocol deviation must be assessed to determine whether it is an important protocol violation. A protocol violation (PV) is important if it affects the rights or safety of the study subjects or if it can potentially influence the primary outcome measure(s) for the respective subjects in a way that is neither negligible nor in accordance with the study objectives. This last category of important PV forms the basis for the decision of whether a subject does or does not belong to an analysis set. PVs that do not influence the subject's rights and safety or the evaluability of the subjects for the main study objectives are called non-important PVs. These are only considered when checking the trial quality in general.

If any important PVs are identified, they are to be summarized into categories and will be captured in the RPM/DBLM minutes via an accompanying Excel spreadsheet [001-MCS-50-413_RD-02] (2). The following table contains the categories which are considered to be important protocol violations in this trial. If the data show other important PVs, this table will be supplemented accordingly by the time of the RPM/DBLM.

Table 6.2: 1 Important protocol violations

Category /Code		Description
A		**Entrance criteria not met**
	A1	Inclusion criteria violated
	A2	Exclusion criteria violated
B		**Informed consent**
	B1	Informed consent not available
	B2	Informed consent too late
C		**Trial medication and randomisation**
	C1	Incorrect trial medication taken
	C2	Randomisation not followed
	C3	Non-compliance
	C4	Incorrect intake of trial medication
	C5	Improper washout between treatments
D		**Concomitant medication**
	D1	Concomitant medication with the potential to affect the assessment of the trial medication
E		**Missing data**
	E1	Certain violations of procedures used to measure primary or secondary data
F		**Incorrect timing**[1]
	F1	Certain violations of time schedule used to measure primary or secondary data
G		**Other trial specific important violations**
	G1	Appropriate fasting condition not met prior to study drug administration
	G2	PVs affecting safety and rights

[1] Time deviations will only be flagged as important PV, when leading to exclusion of the entire subject from an analysis set
Source: 'Protocol Violation Handling Definitions' [001-MCS-50-413_RD-01] (3)

6.3 SUBJECT SETS ANALYSED

- Treated set (TS):
  This subject set includes all subjects who were dispensed study medication and were documented to have taken at least one dose of study drug.
  This is the full analysis set population in the sense of ICH-E9 (1). It is used for safety analyses.

Section 7.3.1 of the CTP: *Plasma concentration data and parameters of a subject will be included in the statistical pharmacokinetic (PK) analyses, if they are not flagged for exclusion due to a protocol violation relevant to the evaluation of PK (to be decided no later than in the Report Planning Meeting) or due to PK non-evaluability (as revealed during data analysis, based on the criteria specified below). Exclusion of a subject's data will be documented in the CTR.*

Relevant protocol violations may be:

- *Incorrect trial medication taken, i.e. the subject received at least one dose of trial medication the subject was not assigned to.*
- *Incorrect dose of trial medication taken.*
- Subject did not receive all study drugs assigned for the specific treatment period.

Plasma and urine concentrations and/or parameters of a subject will be considered as non-evaluable, if for example

- *the subject experienced emesis that occurred at or before two times median t_{max} of the respective treatment (median t_{max} is to be determined excluding the subjects experiencing emesis),*
- *a pre-dose concentration is >5% of the C_{max} value of that subject,*
- *missing samples/concentration data at important phases of PK disposition curve.*

[…]

- *PK parameter analysis set (PKS):*
  The subject set includes all subjects from the TS who provide at least one primary or secondary PK endpoint that was not excluded according to the description above. Thus, a subject will be included in the PKS, even if he contributes only one PK endpoint value for one treatment period to the statistical assessment.

The descriptive analysis of PK concentrations will be based on the ADS ADPC as described at the beginning of Section 7.

Table 6.3: 1 Subject sets analysed

	Subject set	
Class of endpoint	TS	PKS
Safety endpoints	X	
Primary, secondary and further PK endpoints		X
Demographic/baseline endpoints	X	
Important PVs/Disposition	X	
Disposition	X	

6.4 SUBGROUPS

No subgroup analysis is planned.

6.5 POOLING OF CENTRES

This section is not applicable, because the study was performed in only one centre.

6.6 HANDLING OF MISSING DATA AND OUTLIERS

Handling of missing data and outliers will be performed as described in the CTP, Section 7.4.

The only exception where imputation might be necessary for safety evaluation is AE dates. Missing or incomplete AE dates are imputed according to BI standards (see 001-MCG-156_RD-01 (4)).

Missing data and outliers of PK data are handled according to BI standards (see 001-MCS-36-472_RD-01) (5).

6.7 BASELINE, TIME WINDOWS AND CALCULATED VISITS

The baseline value is defined as the last measurement before first trial drug administration in each treatment period, separately for each trial part.

Section 6.1 of the CTP: *Exact times of measurements outside the permitted time windows will be documented. The acceptable time windows for screening and end of trial examination are given in* CTP *Flow Chart .*

Study measurements and assessments scheduled to occur 'before' trial medication administration on Day 1 are to be performed and completed within a 3 h period prior to the trial drug administration.

The acceptable deviation from the scheduled time for vital signs and ECG will be - 15 min after study drug administration on Day 1 and ± 60 min on Day 2.

Adherence to time windows will be checked via the consistency check listings at the RPM/DBLM.

7. PLANNED ANALYSIS

All three trial parts will be evaluated separately.

Safety analysis (refer to Section 7.8) will be performed by and will be presented in Sections 15.1 to 15.4 of the CTR and in Appendix 16.2 and 16.1.13.1.

Inferential statistical analyses of PK endpoints (refer to Sections 7.4 and 7.5.2) will also be performed by and will be presented in Section 15.5 of the CTR and in Appendix 16.1.13.3.

Descriptive data analysis of PK endpoints will be performed by the department Translational Medicine and Clin. Pharmacology at BI and will be presented in Section 15.6 of the CTR.

The format of the listings and tables will follow the standards defined in the BI corporate guideline “Reporting of Clinical Trials and Project Summaries” [001-MCG-159] (6) with the exception of those generated for PK-calculations.

In each trial part, the individual values of all subjects will be listed, sorted by treatment sequence, subject number, visit and actual treatment (if appropriate).
The listings will be included in Appendix 16.2 of the CTR.

For end-of-text tables, the set of summary statistics for non-PK parameters is:

N	number non-missing observations
Mean	arithmetic mean
SD	standard deviation
Min	minimum
Median	median
Max	maximum

For analyte concentrations, as well as for all PK parameters, the following descriptive statistics will additionally be calculated:

CV	arithmetic coefficient of variation
gMean	geometric mean
gCV	geometric coefficient of variation
P10	10th percentile
Q1	1st quartile
Q3	3rd quartile
P90	90th percentile

The data format for descriptive statistics of concentrations will be identical with the data format of the respective concentrations. The descriptive statistics of PK parameters will be calculated using the individual values with the number of decimal places as provided by the evaluation program. Then, the individual values, as well as the descriptive statistics, will be reported with three significant digits in the CTR.

Tabulations of frequencies for categorical data will include all possible categories and will display the number of observations in a category, as well as the percentage (%) for each treatment sequence/group. Percentages will be rounded to one decimal place and will be based on all subjects in the respective subject set, whether they have non-missing values or not. The category 'missing' will be displayed only if there are actually missing values.

Units of variables should be given in the titles or column/row descriptors in square brackets (e.g. [mg]).

Exclusion of PK parameters

The analysis data set (ADS) ADPP (PK parameters) contains column variables APEXC and APEXCO indicating inclusion/exclusion (APEXC) of a PK parameter and an analysis flag comment (APEXCO). All analyses based on the PKS will include parameters if they are not flagged for exclusion; that is, APEXC is equal to “Included”.

Exclusion of PK concentrations

The ADS ADPC (PK concentrations per time-point or per time-interval) contains column variables ACEXC or ACEXCO indicating inclusion/exclusion (ACEXC) of a concentration and an analysis flag comment (ACEXCO). Exclusion of a concentration depends on the analysis flag comment ACEXCO. For example, if ACEXCO is set to ‘ALL CALC’, the value will be excluded for all types of analyses based on concentrations. If ACEXCO is set to ‘DESC STATS’, the value will be excluded from descriptive evaluations per planned time point/time interval. If ACEXCO contains the addition ‘TIME VIOLATION’ or ‘TIME DEVIATION’, the value can be used for further analyses based on actual times. If ACEXCO is set to ‘HALF LIFE’, the value will be excluded from half-life calculation (and, as a consequence, any calculation that relies on λ_z) only; the value is included for all other analyses.

Further details are given in 001-MCS-36-472_RD-01 “Noncompartmental Pharmacokinetic / Pharmacodynamic Analyses of Clinical Studies” (5) and 001-MCS-36-472_RD-03 “Description of Analytical Transfer Files and PK/PD Data Files” (7).

7.1 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

Only descriptive statistics are planned for this section of the report, based on the TS.

The data will be summarised by treatment sequence and in total.

7.2 CONCOMITANT DISEASES AND MEDICATION

Frequency tables are planned for this section of the report, based on the TS.

Concomitant diseases will be coded using the coding system of the Medical Dictionary for Drug Regulatory Activities (MedDRA). Medications will be coded using the World Health Organization Drug Dictionary (WHO-DD). The coding version number will be displayed as a footnote in the respective output.

The diagnoses and medications will be listed. Subjects without any concomitant diagnoses or concomitant therapies should be marked with a "No" in the respective column.

The relevance of the concomitant therapies to the evaluation of PK will be decided no later than at the RPM/DBLM.

7.3 TREATMENT COMPLIANCE

Section 4.3 of the CTP: *Compliance will be assured by administration of all trial medication in the study centre under supervision of the investigating physician or a designee. The measured plasma concentrations and urinary excretion will provide additional confirmation of compliance.*

It is not intended to list the compliance separately. Any deviations from complete intake will be addressed in the RPM/DBLM (cf. TSAP Section 6.2) and described in the CTR.

7.4 PRIMARY ENDPOINTS

Primary analysis

Relative bioavailability is to be determined on the basis of the primary, secondary and some further PK parameters ($AUC_{0\text{-}tz}$, C_{max} and $AUC_{0\text{-}\infty}$, $CL_{R,t1\text{-}t2}$ and $fe_{t1\text{-}t2}$). Those parameters will be ln-transformed (natural logarithm) prior to fitting the ANOVA model (see below).

Section 7.1.3 of the CTP: *The statistical model used for the analysis of primary and secondary* and some further *endpoints will be an ANOVA (analysis of variance) model on the logarithmic scale. This model will include effects accounting for the following sources of variation: 'sequence', 'subjects within sequences', 'period' and 'treatment'. The effect 'subjects within sequences' will be considered as random, whereas the other effects will be considered as fixed. The model is described by the following equation:*

$$y_{ijkm} = \mu + \zeta_i + s_{im} + \pi_j + \tau_k + e_{ijkm}, \text{ where}$$

y_{ijkm} = *logarithm of response (*$AUC_{0\text{-}tz}$, C_{max}, $AUC_{0\text{-}\infty}$*) measured on subject m in sequence i receiving treatment k in period j,*

μ = *the overall mean,*

ζ_i = *the ith sequence effect, i = 1, 2, 3 for part 1 and i = 1,…, 4 for part 2 and 3*

s_{im} = *the effect associated with the mth subject in the ith sequence, m =1, ..., 5 for part 1 and m = 1,..., 4 for part 2 and 3*

π_j = *the jth period effect, j = 1, 2, 3, for part 1 and j = 1,... 4 for part 2 and 3*

τ_k =*the kth treatment effect,* $k \in$ *{R1, T1, T2} for part 1 and* $k \in$ *{R1, T3, R2, T5} for part 2 and* $k \in$ *{R1, T4, R3, T6} for part 3*

e_{ijkm} = *the random error associated with the mth subject in sequence i who received treatment k in period j.*

The difference between the expected means for test treatments and reference treatment ln(T)-ln(R), estimated by the difference in the corresponding Least Square Means (point estimate) and two-sided 90% confidence intervals based on the t-distribution, will be computed. These quantities will then be back-transformed to the original scale to give the point estimator (geometric mean) and interval estimates for the ratio between response under test and response under reference.

The analysis will be accomplished by using the XPKISTAT macro, based on PKS, and option BWC (Bioavailability/Bioequivalence, within-subject design, time-controlled).

Sensitivity analyses

In addition, a sensitivity analysis will be performed by fitting the model described above, but using all effects as fixed. Furthermore, the input dataset will be restricted in such a way that treatments not relevant for the comparison of interest will be deleted.

This analysis will be done using PROC GLM. The following SAS code can be used to fit the model:

```
PROC GLM DATA=indata;
       CLASS subject treatment sequence period;
       MODEL logkp = treatment sequence period subject(sequence);
       LSMEANS treatment / PDIFF=CONTROL("Ref_trt") CL ALPHA=0.1;
RUN;
```

Another sensitivity analysis will analyse the effects within each trial part based on all evaluable data of treatment R1 across the three trial parts. It will be performed by fitting the primary analysis model described above, including 'trial part' as additional (fixed) effect. This analysis will be accomplished by using the XPKISTAT macro, and option BWC, including 'trial part' as covariate (classfix=part).

7.5 SECONDARY ENDPOINTS

7.5.1 Key secondary endpoints

This section is not applicable as no key secondary endpoints have been specified in the protocol.

7.5.2 Secondary endpoints

The same analyses as for the primary endpoints will be performed for the secondary endpoint $AUC_{0-\infty}$ of furosemide, metformin and rosuvastatin.

Section 5.5.1.2 of the CTP: *For digoxin, $AUC_{0-\infty}$ will be evaluated only if determination with sufficient precision is possible.*

7.6 FURTHER ENDPOINTS

Pharmacokinetic (PK)

Secondary analysis

Section 7.1.3 of the CTP: *The secondary analysis will apply the primary analysis model to $CL_{R,t1-t2}$ and to fe_{t1-t2} of each investigated dose of the sensitive drug transporter substrates (digoxin, furosemide, metformin, and rosuvastatin).*

Further analysis

The further analysis will apply the primary analysis model to AUC_{0-tz}, C_{max}, $AUC_{0-\infty}$ and $CL_{R,t1-t2}$ and to fe_{t1-t2} of the therapeutic doses of metformin and furosemide.

Descriptive statistics of PK parameters

Descriptive statistics of plasma and urine concentrations and PK endpoints will be done by the department Translational Medicine and Clin. Pharmacology at BI and will be presented in Section 15.6 of the CTR.

The analysis of PK parameters, as well as the tables and graphs for the pharmacokinetic non-compartmental analyses, will follow specific definitions of this TSAP or, otherwise, the BI standard procedure “Standards and processes for analyses performed within Clinical Pharmacokinetics/Pharmacodynamics” [001-MCS-36-472] (8).

Safety

Refer to TSAP Section 7.8 for a description of the analysis of safety and tolerability.

7.7 EXTENT OF EXPOSURE

Descriptive statistics are planned for this section of the report based on the TS. The date and time of drug administration will be listed for each subject.

7.8 SAFETY ANALYSIS

All safety analyses will be performed on the TS, separately for each trial part.

If not stated otherwise, the safety results will be sorted by actual treatment.

The safety data for treated subjects who failed to complete the study (dropouts or withdrawals) will be reported as far as their data are available. All withdrawals will be documented and the reason for withdrawal recorded.

7.8.1 Adverse events

AEs will be coded with the most recent version of MedDRA.

The analyses of AEs will be descriptive in nature and will be based on BI standards as presented in the corporate guideline: "Analysis and Presentation of Adverse Event Data from Clinical Trials" [001-MCG-156] (9).

The standard AE analyses will be based on the number of subjects with AEs (and not on the number of AEs).

For analysis multiple AE occurrence data on the case report form (CRF) will be collapsed into an AE provided that all of the following applies:

- All AE attributes are identical (lower level term (LLT), intensity, action taken, therapy required, seriousness, reason for seriousness, relationship, outcome, AE of special interest)
- The occurrences were time-overlapping or time-adjacent (time-adjacency of two occurrences is given if the second occurrence started within one hour after end of the first occurrence)

For further details on summarization of AE data, please refer to [001-MCG-156] (9).

No AESIs have been defined for this trial.

The analysis of adverse events will be based on the concept of treatment emergent adverse events.

Section 5.2.2.2 of the CTP: *The REP for digoxin, when measurable drug levels or PD effects are still likely to be present, is defined as 7 days after administration. The REP for the other 7*

probe drugs is shorter. In order to avoid reporting bias (i.e., shorter lengths of on-treatment periods after single component (mono-treatment) administration compared to probe drug cocktail administration could lead to a higher number of AEs after administration of the test treatment), all AEs which occur from first drug administration until 7 days thereafter in each treatment period will be considered as on treatment […].

According to ICH E3 (10), AEs classified as 'other significant' needs to be reported and will include those non-serious and non-significant adverse events with
(i) 'action taken = discontinuation' or 'action taken = reduced', or
(ii) marked haematological and other lab abnormalities or lead to significant concomitant therapy as identified by the Clinical Monitor/Investigator at the Report Planning Meeting.

An overall summary of AEs will be presented.

The frequency of subjects with AEs will be summarized by treatment, primary system organ class (SOC) and preferred term (PT). Separate tables will be provided for subjects with other significant AEs according to ICH E3 (10), for subjects with serious AEs, for subjects with drug-related AEs and for subjects with drug related serious adverse.

The SOC and PTs will be sorted by frequency (within SOC). The MedDRA version number will be displayed as a footnote in the respective output.

In addition, frequencies of subjects with non-serious AEs that had an incidence of > 5% for at least one treatment will be summarised by treatment, primary SOC and PT.

For disclosure of adverse events on EudraCT additional information not included in a standard AE analysis will be performed. The following three entries will be created:

- Adverse Events per arm for disclosure on EudraCT
- Non-serious Adverse Events for disclosure on EudraCT
- Serious Adverse Events for disclosure on EudraCT

7.8.2 Laboratory data

The analyses of laboratory data will be descriptive in nature and will be based on BI standards [001-MCG-157] (11).

Laboratory data will be analysed qualitatively via comparison of laboratory data to their reference ranges. Values outside the reference range as well as values defined as clinically relevant will be highlighted in the data listings.

Possibly clinically significant abnormal laboratory values are only those identified either in the Investigator's comments on the CRF or at the RPM/DBLM at the latest. It is the investigator's responsibility to decide whether a lab value is clinically significant abnormal or not. Standard or project-specific rules for flagging clinically significant values will not be applied in this study.

7.8.3 Vital signs

Descriptive statistics over time including change from baseline will be performed for vital signs (blood pressure and pulse rate).

7.8.4 ECG

12-lead ECG

ECG recordings will be checked by the investigator for pathological results. Clinically relevant abnormal findings for ECG will be listed under 'Relevant Medical History / Baseline Conditions' (when they occurred during screening) or will be reported as AEs (when they occurred during treatment).

7.8.5 Others

Physical examination

Physical examination findings will be reported as relevant medical history/baseline condition (i.e., a condition already existent before intake of study drug) or as AE and will be summarised as such. No separate listing or analysis of physical examination findings will be prepared.

8. REFERENCES

1.	*CPMP/ICH/363/96:* "Statistical Principles for Clinical Trials", ICH Guideline Topic E9, Note For Guidance on Statistical Principles for Clinical Trials, current version.
2.	*001-MCS-50-413_RD-02:* “Important Manual Protocol Violations Spreadsheet”, current version, IDEA for CON.
3.	*001-MCS-50-413_RD-01*: “Protocol Violation Handling Definitions”, current version, IDEA for CON.
4.	*001-MCG-156_RD-01:* "Handling of Missing and Incomplete AE Dates", current version; IDEA for CON.
5.	*001-MCS-36-472_RD-01*: “Noncompartmental Pharmacokinetic/Pharmacodynamic Analyses of Clinical Studies”, current version; IDEA for CON.
6.	*001-MCG-159*: “Reporting of Clinical Trials and Project Summaries”, current version; IDEA for CON.
7.	*001-MCS-36-472_RD-03:* “Description of Analytical Transfer Files and PK/PD Data Files”, current version; IDEA for CON.
8.	*001-MCS 36-472*: "Standards and processes for analyses performed within Clinical Pharmacokinetics/Pharmacodynamics", current version; IDEA for CON.
9.	001-MCG-156: “Analysis and Presentation of Adverse Event Data from Clinical Trials”, current version; IDEA for CON.
10.	*CPMP/ICH/137/95*: “Structure and Content of Clinical Study Reports”, ICH Guideline Topic E3; Note For Guidance on Structure and Content of Clinical Study Reports, current version
11.	*001-MCG-157*: "Handling, Display and Analysis of Laboratory Data", current version; IDEA for CON.

9. ADDITIONAL SECTIONS

Not applicable as no additional information is needed.

10. HISTORY TABLE

Table 10: 1 History table

Version	Date (DD-MMM-YY)	Author	Sections changed	Brief description of change
Final	07-JUN-18		None	This is the final TSAP without any modification