Statistical Analysis Plan

Clinical Trial Protocol Identification No.	BD1604-12CEC MS200585-002
Title:	A phase I, open-label, randomized, three-periods, crossover, partial replicated for the reference drug, single center trial to assess the bioequivalence of a single oral dose of 1200 mg of the new Cisticid® 600 mg tablet formulation *vs* comparator Biltricide® in healthy male volunteers.
Trial Phase	I
Investigational Medicinal Product(s)	Praziquantel
Clinical Trial Protocol Version	2 October 2017/Version 1.0
Statistical Analysis Plan Author	PPD
Statistical Analysis Plan Date and Version	May 2019/Version 1.0
Statistical Analysis Plan Reviewers	PPD ,PPD . PPD ,PPD . PPD ,PPD PPD ,PPD .

1. Signature Page

Statistical Analysis Plan: BD1604-12CEC MS200585-002

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2. Table of Contents

3 List of Abbreviations and Definition of Terms

AE	Adverse Event
AUC	Area under the plasma concentration-time curve
$AUC_{0\rightarrow\infty}$	The AUC from time zero (dosing time) extrapolated to infinity
$AUC_{0\rightarrow t}$	The AUC from time zero (= dosing time) to the last sampling time (t_{last}) at which the concentration is at or above the lower limit of quantification
$AUC_{extra\%}$	The AUC from time t_{last} extrapolated to infinity given as percentage of $AUC_{0\rightarrow\square}$
BE	Bioequivalence
BMI	Body Mass Index
CI	Confidence Interval
$CL_{/f}$	The apparent total body clearance of drug following extravascular administration.
C_{max}	Maximum observed plasma concentration
CRF	Case Report Form
CRO	Contract Research Organization
CSR	Clinical Study Report
CTR	Clinical Trial Report
CTMS	Clinical Trial Management System
CTP	Clinical Trial Protocol
CV	Coefficient of Variation (%)
CYP	Cytochrome P 450
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
GCP	Good Clinical Practice
GeoCV%	Geometric Coefficient of Variation
GeoMean	Geometric Mean
GIR	Glucophage Immediate Release
HR	Hazard ratio

HAV	Hepatitis A Virus
HbA_{1C}	Glycosylated Hemoglobin Type A_{1C}
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IMP	Investigational Medicinal Product
LLOQ	Lower Level of Quantification
Max	Maximum
Mean	Arithmetic mean
Min	Minimum
MedDRA	Medical Dictionary For Regulatory Activities
MRI	Magnetic Resonance Imaging
MSS	Merck Santé s.a.s. in Semoy
N	Number of non-missing observations
PK	Pharmacokinetics
PR	Partial Response
PT	Preferred team
QoL	Quality of Life
SAE	Serious Adverse Event
SASS	Sino-American Shanghai Squibb Pharmaceuticals Ltd
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SD	Standard Deviation
SDTM	Study Data Tabulation Model
SEM	Standard Error of the Mean
SOC	System Organ Class
$t_{1/2}$	Apparent terminal half-life
T2DM	Type 2 Diabetes Mellitus
TEAE	Treatment-Emergent Adverse Event
TLF	Tables, Listings, and Figures

t_{last}	The last sampling time at which the concentration is at or above the lower limit of quantification
t_{max}	The time to reach the maximum observed concentration
TP	Treponema Pallidum
$V_{z/f}$	Apparent volume of distribution during the terminal phase following extravascular administration
λ_z	Terminal elimination rate constant
WHO	World Health Organisation

4 Modification History

Unique Identifier for SAP Version	DATE OF SAP Version	Author	CHANGE DESCRIPTION from the previous version	LEVEL
	May 2019	DRM	New Document	1.0

5 Purpose of the Statistical Analysis Plan

The purpose of this SAP is to document technical and detailed specifications for the final analysis of data collected for protocol BD1604-12CEC MS200585-002. Results of the analyses described in this SAP will be included in the Clinical Study Report (CSR). Additionally, the planned analyses identified in this SAP will be included in regulatory submissions or future manuscripts. Any post-hoc.

The SAP is based upon section 9 (Statistical and Pharmacokinetical Analysis) of the trial protocol and is prepared in compliance with ICH E9.

6 Summary of Clinical Trial Features

6.1 Primary Objectives and Endpoints

- To demonstrate bioequivalence of the fixed combination of praziquantel tablets 600 mg as Cisticid® product compared to Biltricide® tablets 600 mg, given as single dose to 1200 mg, healthy volunteers in fasting state.
- Calculate the primary endpoints AUC0-t, AUC0-∞ and Cmax.

6.2 Secondary Objectives and Endpoints

- The secondary endpoints variable are volume of distribution Ke, half-life elimination, clearance, and median residence time all these parameters will be presented only for informative reasons for both medications.
- To compare the safety and tolerability of all the experimental treatments.

6.3 Overall Trial Design and Plan

This randomized, open-label, single dose, a 3-periods and 3-sequences crossover design with a 7-day wash-out period.
The bioequivalence will be evaluated with primary endpoints are AUC0-t, AUC0-∞ or Cmax for enantiomers and racemic mixture of praziquantel.

The subjects will be randomized to one of the 2 treatment in 3 periods, 3 sequences (see table below). Ten subjects will be randomized to each treatment sequence with a minimum proportion of 30% for each sex in each treatment sequence.

Sequence	Period 1	Wash-out period	Period 2	Wash-out period	Period 3
PRR	Cisticid®		Biltricide®		Biltricide®
RPR	Biltricide®		Cisticid®		Biltricide®
RRP	Biltricide®		Biltricide®		Cisticid®

7 Sample Size

The sample size estimation is based on the CV intrasubject variability of praziquantel.

The sample size is determined by the variability currently available, these values are 60 to 50 and 35% for Cmax and AUC, respectively. With this information, praziquantel is considered as a drug highly variable. In a clinical investigation realized by Merck Serono by a phase 1 design the variability obtained was 34.5% for L-praziquantel, 34.5% for AUC and 52% for Cmax.

The sample size calculation was realized using the PPD functions recommended by European Medicines Agency (EMA).

Considerations

- The reference-scaled average bioequivalence is applicable only for Cmax, for ABC apply the c conventional bioequivalence limits
- If the CVintrasubject value is greater than 50% the bioequivalence limits will be 0.70-1.43

Several assumptions were considered, there are:

- The real mean of ratio test/reference for primary pharmacokinetic parameters.
- Cmax CVintra subject reference: 45-55%
- Cmax CVintra subject test: 45-55%
- AUC CVintra subject reference: 39.5%
- AUC CVintra subject test: 39.5%
- Alpha value: 0.05
- Power 80%
- Droup-out rate: 20%

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With all these assumptions the sample size required is 48 healthy subjects are needed to obtain power greater of 80%, neverthless considering the 20% of rate droup-out the sample size needed to avoid this problem is 60 subjects.
The 60 subjects going to be randomized into 3 sequences for each treatment, for this reason 20 subject will be enrolled in each treatment sequences.

8 Overview of Planned Analyses

PK parameters will be performed using PPD [redacted]. Statistical analysis will be performed using SAS 9.4.

Descriptive statistical methods will be used to summarize demographic characteristics, pharmacokinetics parameters and adverse events. After the data base closing

Individual plasma concentration-time will be tabulated and plotted. Primary pharmacokinetics parameters, AUC and Cmax, will be tabulated and plotted by subject. Likewise, differences and odd ratios for test/reference will be tabulated for each subject for those parameters. Plasma concentration - time graphs will be made using arithmetic and semi-logarithmic scale. All de calculations will be determinated in concordance with NOM-177-SSA1-2013

Bioequivalence test methodology will be perform using the next codes:
PRC GLM DATA=PK
CLASS SEQ SUBJ PER TRT;
MODELLNPARAM=SEQ PRE TRT SUBJ(TRT)/DDFM=SATTERTH;
LSMEANS TRT/ADJUST=T PDIFF CL ALPHA=0.01.
TEST H=SEQ E=SUBJ(TRT);
RUN;
QUIT;

8.1 Interim Analysis

Bioequivalence for AUC:

A mixed model will be applied to log-transformed AUC0-t and AUC0-∞ with treatment, period and sequence as fixed effects, and subject (sequence) as a random effect. Based on the residual error term 90% confidence intervals will be computed for the estimated differences Test – Reference, resulting in 90% confidence intervals for the Test/Reference ratios after back-transformation.

Bioequivalence for Cmax:
The 90% confidence intervals will be computed for the estimated differences Test – Reference. If $CV_{WR} \leq 30$ the procedure to estimate is the same as the confidence intervals for AUC, if $30\% < CV_{WR} \leq 50\%$, the acceptance interval will be widened according to the next formula (U, L) = exp(±k'sWR), where U is the upper limit, L is the lower limit, k is the constant established as 0.760 and sWR is the intrasubject standard deviation for the Cmax log transformated value for reference product. For values greater than 50% the aceptace range going to be 69.84 – 143.19

TO evaluate the group effect, the next codes will be used:
PROC GLM DATA=PK

```
CLASS SEQ SUBJ PER TRT GROUP;
MODEL LNPARAM=SEQ PER TRT GROUP GROUP*TRT SUBJ(TRT)/ DDFM=
SATTERTH;
LSMEANS TRT/ ADJUST=T PDIFF CL ALPHA=0.10.
TEST H=SEQ E=SUBJ(TRT);
RUN;
QUIT;
```

Statistical analysis for secondary parameters
The secondary pharmacokinetics parameters (Tmax, AUCextrapolated, t½, Cl/F and Vd/F) going to be analyzed by descriptive statistics

9 Changes to the Planned Analyses in the Clinical Trial Protocol

The statistical methods as described in the protocol were adopted.

There are no changes to the planned analyses.

10 Protocol Deviations and Analysis Sets

10.1 Definition of Protocol Deviations and Analysis Sets

All deviations should be justified with statistical or scientific data and any change to the original statistical plan should be documented, in the study master file and in the pharmacokinetics statistical report as well as in the final study report. Subjects' data will not be replaced. Any missing data will be considered as non-existent data. Likewise, data cannot be removed from the statistical analysis, except in the following events.

- **Research Subjects with Pre-dose Concentrations in the Biological Matrix**

In the event pre-dose concentration is < 5 % of the C_{max} value for a research subject, subject's data can be included without any adjustment to measurement and pharmacokinetic calculations. When pre-dose value is > 5 % of the C_{max}, research subject should be removed from all study bioequivalence assessments.

- **Exclusion of data due to vomit or diarrhea.**

Data from research subjects who experience vomiting and diarrhea throughout the bioequivalence study for immediate release products can be removed from the statistical analysis if vomiting and diarrhea occur before 2 fold the median for t_{max} or 2 fold t_{max} value obtained from the research subject in a given period.

- **Research subject with very low plasma concentrations for study drugs.**

As established by NOM-177-SSA1-2013, research subjects in a cross designed who provide evaluable data for test drug and reference drug, or who do not have evaluable data in the single period of a parallel design, should not be either included in the statistical analysis.

It is considered that a research subject has very low concentrations, if the AUC is lower than 5 % of the geometric means for the reference drug's AUC (it should be calculated without including outliers). Exclusion of data due to this reason will only be accepted prior scientific rationale and review of the case by the COFEPRIS.

11 General Specifications for Statistical Analyses

Pharmacokinetic parameters (non-compartmental analysis) and statistical analysis for determining bioequivalence will be calculated using Phoenix WinNonlin 8.0 software.
The results of this trial will be reported using summary tables, figures, and data listings, as appropriate. All data will be summarized by treatment and/or scheduled time point, as appropriate.

12 Protocol Deviations

12.1 Important Protocol Deviations

Not applicable. No deviation was observed in the study

12.2 Reasons Leading to the Exclusion from an Analysis Set

- **Research Subjects with Pre-dose Concentrations in the Biological Matrix**

In the event pre-dose concentration is less than 5 % of the C_{max} value for a research subject, subject's data can be included. When pre-dose value is greater than 5 % of the C_{max}, research subjects data should be removed from all study of food effect on bioavailability.

- **Exclusion of data due to vomit or diarrhea.**

Data from research subjects who experience vomiting and diarrhea throughout the study for immediate release products can be removed from the statistical analysis if vomiting and diarrhea occur before 2 fold the median for t_{max} or 2 fold t_{max} value obtained from the research subject in a given period.

- **Research subject with very low plasma concentrations for study drugs.**

As established by NOM-177-SSA1-2013, research subjects in a cross designed who provide evaluable data for test drug and reference drug, or who do not have evaluable data in the single period of a parallel design, should not be either included in the statistical analysis.

It is considered that a research subject has very low concentrations, if the AUC is lower than 5 % of the geometric means for the reference drug's AUC (it should be calculated without including outliers). Exclusion of data due to this reason will only be accepted prior scientific rationale and review of the case by the COFEPRIS.

13 Demographics and Other Baseline Characteristics

Demographic data from each volunteer is shown in table 13.1 and the descriptive statistics for the demographic variables from volunteers recruited in the study are shown in table 13.2. The sample size stated in the clinical protocol was of 22 subjects from both genders. Randomization was performed with the 22 subjects proposed in the clinical protocol.

Subject 11 was removed because she received and took food, nevertheless, she was in fasting condition sequence, for this reason, she was changed to 22 case (fed conditions). The statistical final analysis was made with 21 subjects. The percentage of observations were 99.84% from 1848 plasmatic concentrations, missing observations were only 0.16%, 3 from 1848.

13.1 Demographics

Table 13.1. Demographic Variables' Individual Data

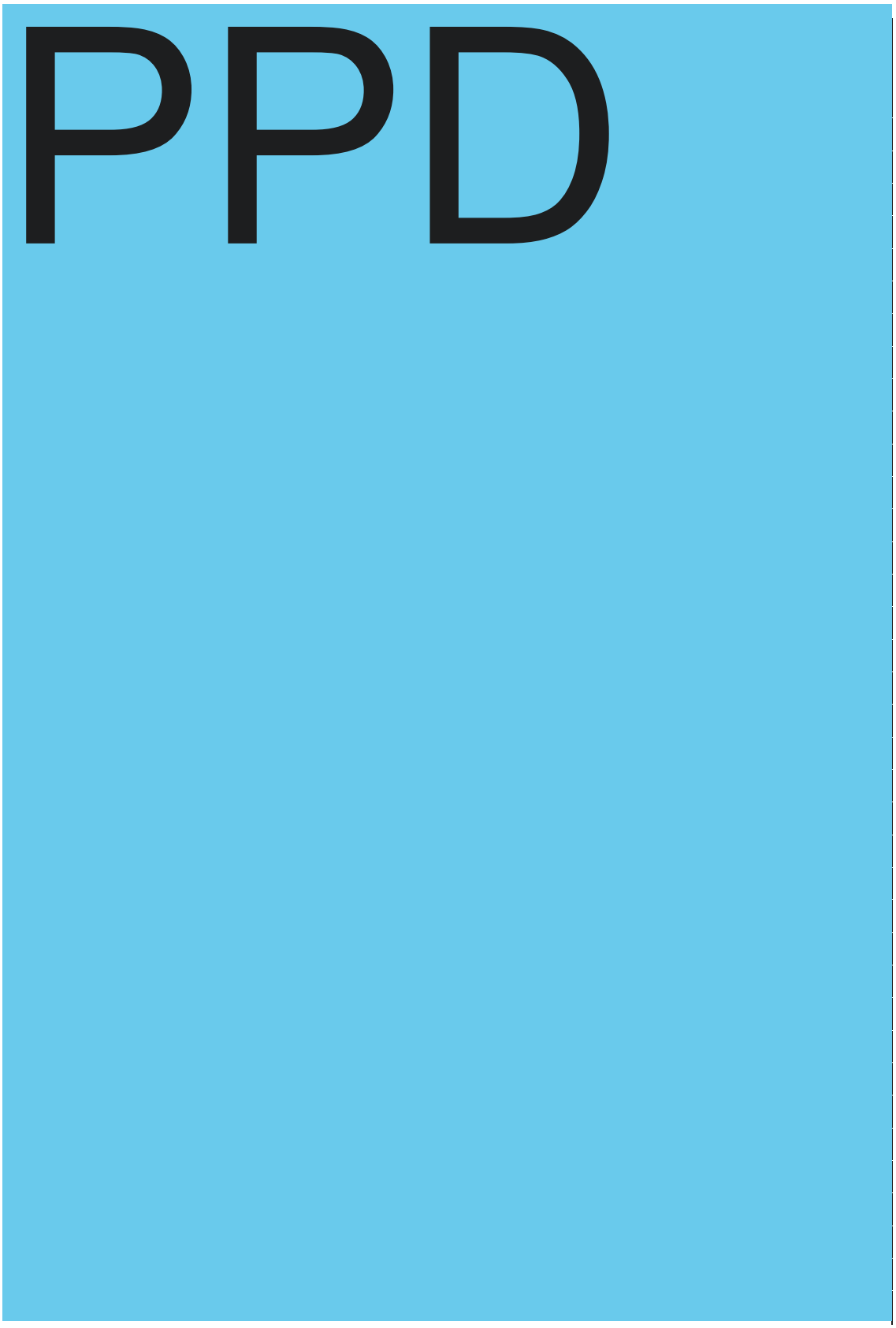
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Table 13.2. Demographic Variables' Descriptive Statistics

14 Treatment Compliance and Exposure

All subjects receive the investigational treatment at the pre-specified fixed dosage. Information relating to the extent of exposure is thus contained in the treatment labelling.

15 Endpoint Evaluation

15.1 Primary Endpoint Analyses

The determination of pharmacokinetics parameters were determined using WinNonlin software depending of their own characteristics as follows:

Cmax: is the peak or maximum concentration

AUC_{0-t}: area under de curve computed from time zero to the time of the last positive Y value.

$AUC_{0-\infty}$: area under de curve computed from time zero to extrapolated from infinity.

Ke: First-order rate constant associated with the terminal (log-linear) elimination phase. This is estimated via linear regression of time vs. log concentration.

15.2 Bioavailability Statistics

In tables 15.2.1 and 15.2.2, pharmacokinetic equivalence statistical results are shown for the metformin's logarithmically transformed pharmacokinetic data, for L-praziquantel and racemic mixture

Table 15.2.1. Statistics for the L-enantiomer of praziquantel

Table 15.2.2. Statistics for the racemic mixture of praziquantel

Table 15.2.1 shows the results of the evaluation of L-enantiomer. Results show that there is pharmacokinetic equivalence, all the results are included in acceptance ranges

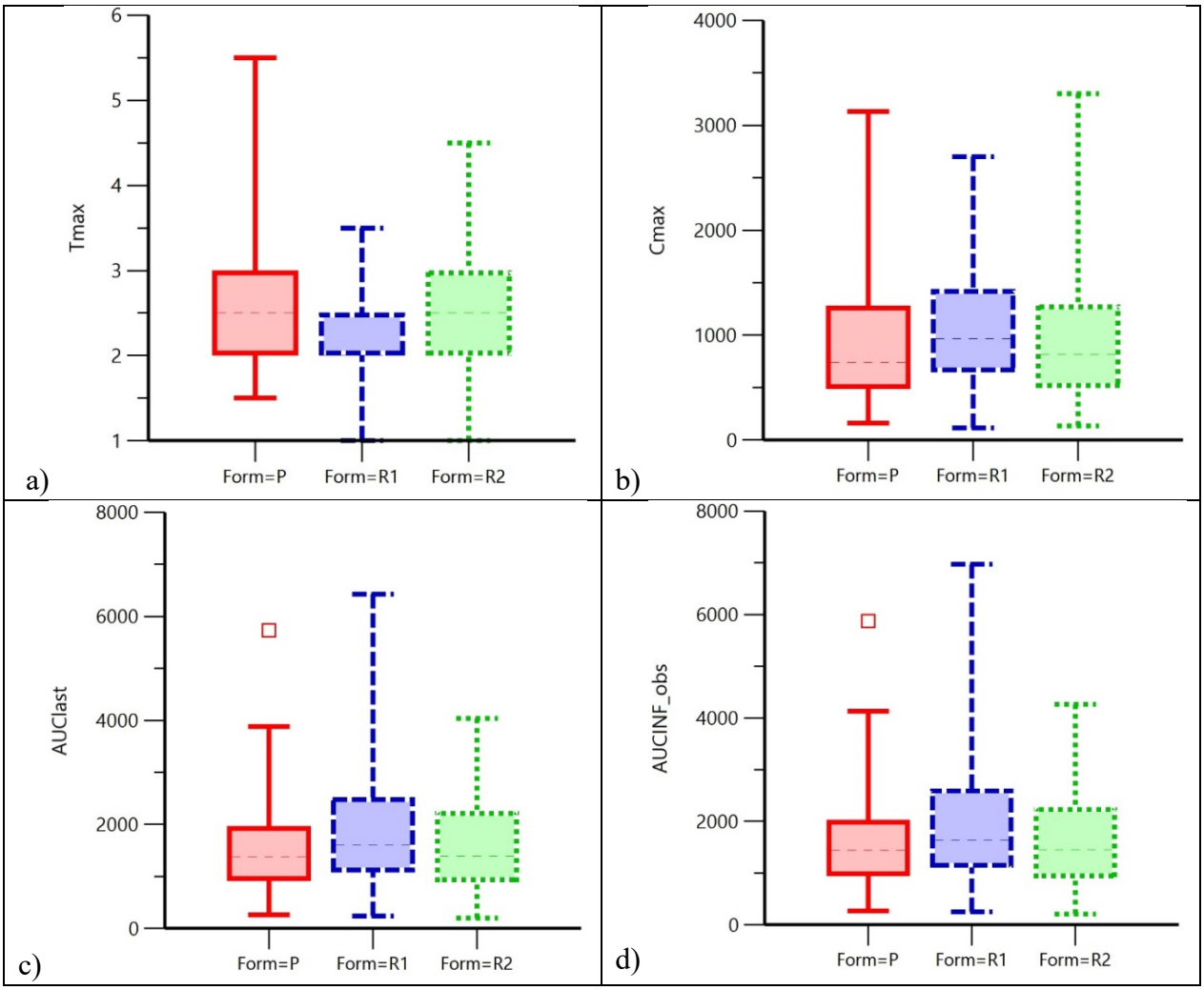
In table 15.2.2, shows the results of the evaluation of racemic mixture. Results show that there is pharmacokinetic equivalence, all the results are included in acceptance ranges

With the results of the pharmacokinetic equivalence statistics, it might be concluded that there are not statistical significances with both analytes.

15.3 Box and Whiskers plots.

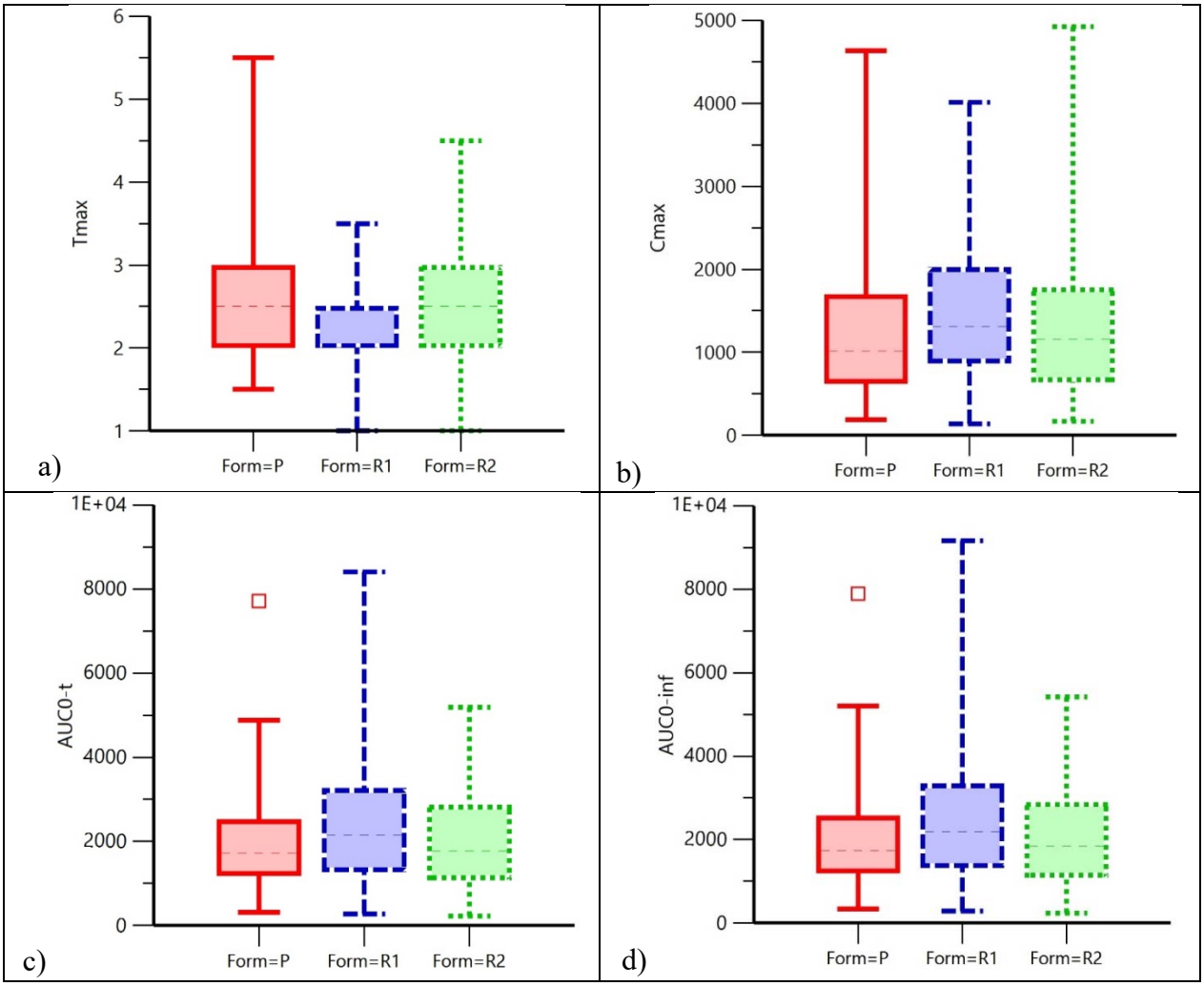
In tables 15.3.1 and 15.3.2, box and whiskers plots corresponding to the parameters used to assess the absorption and bioavailability for L-praziquantel and racemic praziquantel, respectively, are shown. Graphically it is confirmed that there are not differences between the reference drug's dosing and the test drug for L-praziquantel, while there are no differences for racemic mixture.

Figure 15.3.1 Box and Whiskers Plot for the L-praziquantel Pharmacokinetic Parameters



a) Plot for the t_{max} parameter, b) Plot for the C_{max},
c) Plot for the AUC_{0-t}; and d) Plot for the $AUC_{0-\infty}$.
Form P is the Cisticid® test formulation, form R1 is Biltricide® first administration and formulation R2 is Biltricide® second administration

Table 15.3.2. Box and Whiskers Plot for the racemic mixture of praziquantel Pharmacokinetic Parameters



a) Plot for the t_{max} parameter, b) Plot for the C_{max},
c) Plot for the AUC_{0-t}; and d) Plot for the $AUC_{0-\infty}$.
Form P is the Cisticid® test formulation, form R1 is Biltricide® first administration and formulation R2 is Biltricide® second administration

As shown in figure 15.3.2, the t_{max} parameter has no differences in the variation following

15.4 Secondary Endpoint Analyses

Average results of the L-praziquantel enantiome pharmacokinetic parameters are shown in tables 15.4.1, in table 15.4.2 are shown the pharmacokinetics results for racemic mixture.

Table 15.4.1. L-praziquantel Pharmacokinetic Parameters Results

Table 15.4.2. Racemic mixture Pharmacokinetic Parameters Results

15.5 Other Endpoint Analyses

Data about plasma concentration versus time for each subject, as well as the descriptive statistics are shown in tables 15.5.1 for L-praziquantel and in table reference product in first administration, 15.5.2 for L-praziquantel and in table reference product in second administration, 15.5.3 for L-praziquantel and in table test product.

Table 15.5.1. Individual concentrations table corresponding to L-praziquantel. Reference product in first administration. n = 59 volunteers

NA: Not applicable.

Table 15.5.2 Individual concentrations table corresponding to L-praziquantel. Reference product in first administration. n = 59 volunteers

NA: Not applicable.

Table 15.5.3 Individual concentrations table corresponding to L-praziquantel. Test product. n = 59 volunteers

NA: Not applicable.

As can be seen in tables 15.5.1, 15.5.2 and 15.5.3 at time 0.00 h, plasma concentration values for metformin from all subjects was 0.0000 ng/mL in both dosing states, which shows that the wash-out period was appropriate.

Data about plasma concentration versus time for each subject, as well as the descriptive statistics are shown in tables 15.5.4 for racemic mixture reference product in first administration, 15.5.5 for racemic mixture reference product in second administration and 15.5.6 for racemic mixture test product.

Table 15.5.4. Individual concentrations table corresponding to racemic mixture. Reference product in first administration. n = 59 volunteers

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-: Sample not delivered.

Table 15.5.5 Individual concentrations table corresponding to racemic mixture. Reference product in second administration. n = 59 volunteers

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NA: Not applicable.

Table 15.5.6 Individual concentrations table corresponding to racemic mixture. Test product. n = 59 volunteers

Average values for the metformin and gliclazide's concentration obtained in the study as well as the descriptive statistics are shown in tables 15.5.7 and 15.5.8.

Table 15.5.7.. Descriptive Statistic for the Praziquantel S enantiomer´s Concentration Values (ng/mL) in Biological Fluid

Table 15.5.8 Descriptive Statistic for the Praziquantel Racemic Mixture´s Concentration Values

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[redacted]

16. Estimation of Individual Pharmacokinetic Parameters

Non-compartmental computation of pharmacokinetic parameters was performed using the computer program Phoenix® WinNonlin® version 8.0 (PPD).

The pharmacokinetic analysis was performed with a non-compartmental analysis, using real times following the dosing of the studied drugs. Pharmacokinetic parameters were determined as shown in tables 16.1 for Cmax, 16.2, for AUC_{0-t} and 16.3 for $AUC0_{-\infty}$ for L-praziquantel. The tables 16.4 for Cmax, 16.5, for AUC_{0-t} and 16.6 for $AUC0_{-\infty}$ for racemic mixture, respectively.

Table 16.1. Individual Data and Descriptive Statistics for L-praziquantel, C_{max}.

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Table 16.2. Individual Data and Descriptive Statistics for L-praziquantel, AUC_{0-t}.

Table 16.3. Individual Data and Descriptive Statistics for L-praziquantel, $AUC_{0-\infty}$.

Table 16.4. Individual Data and Descriptive Statistics for racemic mixture, C_{max}.

Table 16.5. Individual Data and Descriptive Statistics for racemic mixture, AUC_{0-t}.

Table 16.6. Individual Data and Descriptive Statistics for racemic mixture, $AUC_{0-\infty}$.

17 Safety Evaluation

According to the Mexican Official Standard, NOM-177-SSA1-2013, there are several statistical tests to identify extreme values. Most of them start by calculating the student residual absolute value. Likewise, it is stated that "since studies are generally crossed designed, the most important extreme values is the extreme value for the subject".

An adequate method for estimating extreme values allows increasing reliability of the study conclusion. An analysis to identify outliers (extreme) values based on the student residual estimation among subjects will be performed using Bear software (current) for R environment.

Criterion: extreme values are those data which degree is higher than ± 2 standardized residuals intra-subject.

18. Adverse Events

All the information related with clinical results is included in clinical inform

19 References

- Chow S.S, Liu JP. (2009). Design and Analysis of Bioavailability and Bioequivalence Studies. 3rd edition. US: CRC Press.
- Mexican Official Standard NOM-177-SSA1-2013 which sets out the tests and procedures to prove that a drug is interchangeable, requirements for the authorized third parties performing interchangeability tests; requirements for the conduct biocomparability studies, requirements for authorized third parties, research centers and hospitals conducting biocomparability tests.

20 Appendices

- Quality Assurance Report.
- Individual Concentrations
- Winnonlin Core Outputs