Clinical Trial Protocol

Clinical trial protocol number	BD1412-63CEC EMR200763-004
Title	A randomized, open-label, single dose, 2x2 crossover trial to evaluate the food effect on the bioavailability of a Metformin/Gliclazide fixed combination tablet (1000 mg / 30 mg MR) given in fasting and fed state to healthy volunteers.
International non-proprietary name	Metformin/Gliclazide.
Phase	Phase I (food interaction)
IND Number	not applicable
EudraCT Number	not applicable
Principal Investigator	PPD
Sponsor	Merck KGaA Darmstadt. Name: PPD Address Frankfurter Strasse 250 64293 Darmstadt, Germany (phone)|
Study Monitor	PPD
Date of elaboration and version	Nov/15/2017, version 2.0

Clinical Unit

Name:	PPD	Signature:	
Role:	Principal Investigator	Date:	
Name:	PPD	**Signature:**	
Role:	Sanitary Officer	**Date:**	
Name:	PPD	**Signature:**	
Role:	Quality Assurance Officer	**Date:**	
Address:	PPD		
Telephone:	PPD /Fax: PPD		

Analytical Unit

Name:	PPD	**Signature:**	
Role:	Bioanalytics Investigator	**Date:**	
Name:	PPD	**Signature:**	
Role:	Sanitary Officer	**Date:**	

Name:	PPD	**Signature:**	
Role:	Responsible for Biostatistics	**Date:**	
Name:	PPD	**Signature:**	
Role:	Quality Manager	**Date:**	
Address:	PPD		
Telephone:	PPD		

S p o n s o r

Name:	PPD	**Signature:**	
Role:	Responsible for the Study	**Date:**	

C o m m i t t e e s

Ethics on Investigation Committee	PPD
Investigation Committee:	PPD

C l i n i c a l L a b o r a t o r y

Clinical Laboratory:

PPD

Ph. PPD

Table of contents

Abbreviations list

µg	Microgram
A	Treatment in fasted state
A´	Treatment in fed state
AE	Adverse Event
AUC	Area under the plasma/serum - concentration time curve
$AUC_{0\rightarrow\infty}$	Area under the plasma/serum concentration time curve from 0 to extrapolated to infinity
$AUC_{0\rightarrow t}$	Area under the plasma/serum concentration time curve from 0 to last time of sampling
BMI	Body Mass Index
CARPERMOR	PPD
CBC	Complete Blood Count
CL/f	Apparent total body clearance of drug from plasma
C_{max}	Maximum Plasma Concentration
CNFV	Mexican National Center of Pharmacovigilance
COFEPRIS	Federal Commission for the Protection against Sanitary Risks
CRF	Case Report Form
DBP	Diastolic Blood Pressure
dL	Deciliter
E	Elimination
ECG	Electrocardiogram
FDA	Food and Drug Administration (U.S.)
g	grams
GCP	Good Clinical Practice
h	Hour
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference of Harmonization
IUPAC	*International Union of Pure and Applied Chemistry*
IV	Intravenous
K_e	Elimination Rate Constant

min	Minute
mL	Milliliter
MoH	Ministry of Health
MRT	Mean Residence Time
PI	Principal Investigator (Study Coordinator, Clinical Unit)
PT	Prothrombin time
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SD	Standard Deviation
SOPs	Standard Operating Procedures
t	Time
t½	Elimination Half Time
t_{max}	Time of peak plasma concentration
TPT	Partial thromboplastin time
Vz/f	Apparent volume of distribution

1 Summary

Protocol number	**BD1412-63CEC** **EMR200743-004**
Title	A randomized, open-label, single dose, 2x2 crossover trial to evaluate the food effect on the bioavailability of a Metformin/Gliclazide fixed combination tablet (1000 mg /30 mg MR) given in fasting and fed conditions to healthy volunteers.
Phase	Phase I (food interaction)
IND Number	Must match title page or indicate 'not applicable'
FDA covered trial	Indicate 'yes' or 'no'
EudraCT Number	Must match title page or indicate 'not applicable'
Principal Investigator	PPD
Sponsor	Merck KGaA
Sponsor's legal representative at the European Union	Merck KGaA
Investigational sites/Countries	Not applicable
Scheduled study period (first subject and last subject)	Not applicable
Study approval	COFEPRIS

Objectives:

Primary objectives

To assess the food effect on bioavailability ($AUC_{0-\infty}$, AUC_{0-t} and C_{max}) for the fixed dose combination Metformin 1000 mg/Gliclazide 30 mg MR, given as single dose in fasted state and with food to healthy volunteers.

Secondary objectives

To determine further pharmacokinetic parameters such as t_{max}, $t_{1/2}$, Vz/f and Cl/f.

To assess the safety and tolerability of the fixed dose combination in fed and fasted state

Methodology: A randomized, open label, single dose, 2 period crossover design will be used with 22 subjects in fed and fasted state, with a 14-day washout period between 2 study stages.

Planned number of subjects: 22 (at least 40 % for each gender by study group).

Primary variable: the variables going to be $AUC_{0-\infty}$, AUC_{0-t} and C_{max} for Metformin and Gliclazide, in fed and fasted state.

Secondary objectives:

- Pharmacokinetic parameters will be determined: t_{max}, Vz/f and CL/f.
- To assess and compare the safety and tolerability of the fixed dose combination Metformin /Gliclazide administered in fasted and fed state.

Pharmacokinetic parameters: Main parameters will be AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} for Metformin and Gliclazide.

Key inclusion and exclusion criteria:
Inclusion criteria:

Subject grants informed consent prior any study related activity will be performed.

1. Ethnicity: Mexicans (e.g., Caucasian, native people and Mestizo [mixed race])
2. Gender: Female and male (at least 40 % for each gender by study group).
3. Age between 18 and 55 years old, both including.
4. Weight between 55 and 95 kg.
5. Body mass index between 18 and 27 kg/m^2.
6. Nonsmokers or subjects who do not smoke more than 5 cigarettes or 1 pipe a day
7. Good physical and mental health based on the clinical history and physical examination.
8. All results from blood chemistry, hematology, and urinalysis should be within normal ranges or without clinically significant deviations as per Principal Investigator's judgment*.
9. Hematology complete blood count [CBC]: hematocrit and hemoglobin must be above the lower limit; upper limit may range up to 15 %. Remaining results, including white blood cells may range ± 15 %, if subject is asymptomatic.
10. Liver Function Test, including direct and indirect bilirubin, glutamic oxaloacetic transaminase (aspartate aminotransferase) and glutamic pyruvic transaminase (alanine aminotransferase), total proteins, albumin, globulin and albumin/globulin ratio. Total bilirubin from 0.00 to 1.6 mg/dL, direct bilirubin from 0.0 to 0.4 mg/dL and indirect bilirubin from 0.0 to 1.2 mg/dL. They may range up to 15 % the enzyme's upper limit. Lower limit has no restriction for subjects' inclusion.
11. Electrocardiogram (12 leads) without clinically significant pathological signs, particular in QTc interval Bazett (normal value < 450 ms).
12. All women of childbearing potential must have negative tests for pregnancy (qualitative and quantitative) at screening, and at day -1 for each treatment period and at EOT as described in section 8.5.3.
13. Vital signs (blood pressure and pulse) in supine position within normal ranges or with clinically significant abnormalities as per the Principal Investigator's judgment.
    a. Heart rate between 50 and 100 beats per minute.
    b. Respiratory rate between 12 and 20 per minute.
    c. Systolic blood pressure between 80 and 129 mmHg.
    d. Diastolic blood pressure between 50 and 89 mmHg.
    e. Forehead temperature between 36.0 and 37.0 °C.

14. All women of childbearing potential who are not pregnant or breastfeeding and who are using a highly effective contraceptive method (defined as those, alone or combined, which failure rate is low; i.e., less than 1 % a year, when used continuously and correctly) for at least one month before and following dosing. Barrier methods and intrauterine device are considered standard contraceptive methods. Hormonal methods will not be included. Post-menopausal women can be included (i.e., those with at least 12 consecutive months of amenorrhea following their last menstruation period) or surgically sterile/hysterectomy for at least 6 months before their participation in the study.

15. Negative result for alcohol breath test and urine test for drugs of abuse (opioids, barbiturates, cocaine and its metabolites, amphetamines, metamphetamines, morphine, cannabinoids, benzodiazepines and tricyclic antidepressants), at screening and at each day -1 of the 2 treatment periods.

16. Negative serology tests for human immunodeficiency virus (HIV1 and HIV2 antibodies), hepatitis A (HAV), hepatitis B (HBV), hepatitis C (HCV) and VDRL test screening.

Exclusion criteria:

1. Subjects who have received any investigational drug within 21 days prior to the study start.

2. Subjects who have donated or lost 450 mL or more of blood within 21 days prior to the study start.

3. Subjects with history of cardiovascular, renal, liver, metabolic, gastrointestinal, neurological, endocrine, or hematopoietic (any type of anemia) diseases; mental disease, surgery or other organic abnormalities which might affect the study of the investigational drug pharmacokinetics.

4. History of gastrointestinal tract surgery that might affect gastrointestinal absorption and/or motility as per the investigator's judgment.

5. Subjects with history of hypersensitivity to the study drug and/or any formulation's ingredient; history of drug induced anaphylaxis.

6. Subjects who take any other drug, prescribed or over the counter drugs 30 days before the study drug dose and for which at least seven elimination half-lives had not elapsed, including multi-vitamins and herbal products (e.g., St. John's Wort) including ASA and oral contraceptives for women. Except for paracetamol (maximum 1000 mg per day for three consecutive days) that can be given if required, as per the Principal Investigator's judgment.

7. Renal failure or renal impairment (creatinine clearance < 80 mL/min), assessed by using the Cockcroft-Gault formula.

8. Subject's disagreement or lack of capacity to communicate and cooperate with the Investigator, lack of legal capacity or limited legal capacity which prevent him/her from continuing in the study.

9. Refusal of the high-fat diet which is necessary to assess the food effect. Considerable deviations to the diet's normal nutritional patterns.

10. Subjects who have smoked tobacco, having drunk alcohol, or xanthines containing beverages or food (coffee, tea, cacao, chocolate, mate, coke drinks, etc.) above 600 mg of caffeine a day (a 240-mL cup of coffee contains 100 mg of caffeine, one cup of tea contains 30 mg and a glass of coke contains 20 mg of caffeine); those who have had grilled food (charcoal) within 24 h prior to the drug dosing (*Fuhr 1998*).

11. Intake of grapefruit, orange, cranberries or their juices within 14 days prior to the drug's dosing and throughout the study.

12. Legal inability or limited legal capacity

13. Incarcerated subjects.

14. Subjects who have been exposed to agents known as liver enzyme systems' inducers or inhibitors, or who have taken potentially toxic drugs within 30 days prior to the study.

Study drug: dosage/route/dosing schedule:

Fixed dose of Metformin 1000 mg/Gliclazide 30 mg MR (one tablet), single dose, given in fasting or postprandial state.

Study duration: Approximately seven weeks

Statistical method: According to the goals for the study, treatment regarding postprandial/fasting, pharmacokinetic parameters will be calculated AUC and C_{max} for both analytes, Metformin and Gliclazide. In order to have an accurate and reasonable precision for the estimated ratios, a sufficient amount of subjects will be randomized. Taking 90 % confidence interval for the postprandial/fasting ratio as a measure for accuracy and assuming an average variation coefficient of 20 % for AUC ($AUC_{0-\infty}$, AUC_{0-t}) and C_{max} for Metformin or Gliclazide, then with 18 evaluable subjects the food effect on pharmacokinetics can be estimated with an accuracy of 12 %.

The following ratio will be carried out, being R the estimated ratio and LCL/UCL lower and upper confidence limit for the 90 % confidence interval for R:

$0.89 * R \leq LCL < UCL \leq 1{,}12 * R$

An accuracy of 12 % or higher will result in an accurate description for the food effects. Besides, 18 evaluable subjects are enough to detect a difference among treatments of 18 % or higher with a power of 80 % at least. Taking into account a drop-out rate around 20 %, 22 subjects should be randomized.

This trial intends to estimate the food effects on the pharmacokinetics. It is not intended to prove or confirm statistically the lack of food effect.

Therefore, no statistical hypothesis is shown. As primary analysis, a mix model for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ log-transformed will be applied. With TREATMENT, PERIOD and SEQUENCE as fixed effects, and the SUBJECT (SEQUENCE) as random effect. Based on residual errors term, confidence intervals of 90 % will be built for estimated differences for postprandial - fasting, resulting in confidence intervals of 90 % for the postprandial/fasting ratios following transformation.

2 Sponsor, Principal Investigator and Study Administrative Staff

The study will be sponsored by: Merck KGaA. Frankfurter Strasse 250 64293 Darmstadt, Germany

The study will be conducted in one site, PPD .

The Principal Investigator, PPD from the PPD , represents all investigators with regards to decisions and discussions related to the study according to International Conference on Harmonization (ICH), topic E6 from the Good Clinical Practice (GCP, referred as ICH GCP in the following.) The Principal Investigator will provide expert physicians and advice with regards to the trial design and conduct. PP is responsible for the review and approval of the clinical study report.

This protocol meets requirements of current clinical research regulations, including: GCP's, ethical principles for clinical research in human beings from the Declaration of Helsinki issued in the 64th General Assembly from the World Medical Association, Fortaleza, Brazil, October 2013; General Health Act and General Health Act Regulations in the field of Health Research.

The study will be listed in the following registries for clinical trials: National Registry for Clinical Trials from the Mexican Ministry of Health through the Federal Commission for the Protection against Sanitary Risks.

Study Monitor: PPD . PPD .

3 Background

This study will be conducted according to the provisions stated in NOM-177-SSA1-2013 which states that "tests and procedures to prove that a drug is interchangeable as well as the requirements which authorized third parties, performing tests, should adhere", including the Good Clinical Practice (GCPs), ICH and all other applicable regulations.

According to the General Health Act Regulations in the Field of Health Research, second title, chapter I, article 17, section III which was published on the Official Gazette on April 2nd, 2014 this study is considered an investigational study with risk higher than minimum.

Based on the non-clinical and clinical data available so far, the conduct of the trial specified in this clinical trial protocol is justified.

Refer to the Investigator's Brochure (IB) to get more information about programs and non-clinical and clinical advice for the Investigator.

3.1.1 Metformin's Pharmacology

Systematic name as per IUPAC for Metformin is N, N-dimethylmidodicarbonimidicyl Diamide.

Metformin empiric formula is $C_4H_{11}N_5$. Metformin's molecular weight is 129.164 g/mol and Metformin hydrochloride is 165.63 g/mol (Pubchem, 2015.)

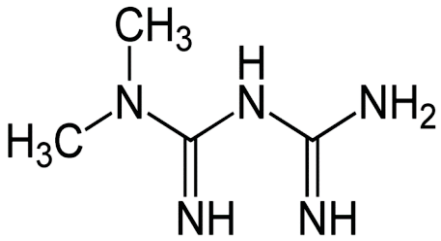

Figure 1. - Metformin

3.1.2 Absorption

Metformin's apparent distribution volume (Vz/f) following oral single dose of Metformin HCl 850 mg tablets averaged 654 ± 358 L. Metformin has a minimum bound to proteins. At usual clinical doses and dosage of Metformin hydrochloride, plasma concentration in steady state is achieved within 24-48 h and generally is < 1 mg/mL (Green and Feinglos, 2008, Cho et al., 2009). Following the oral dose of Metformin XR 500 mg, T_{max} was of 7 hours and C_{max} of 645 (115) ng/mL.

3.1.3 Distribution

Maximum concentrations are lower in blood than in plasma and are achieved approximately simultaneously. Erythrocytes seem to represent the secondary distribution compartment. Mean distribution volume following IV administration is between 63 and 276 L.

It is quickly distributed to tissues and peripheral body fluids. It is slowly distributed in erythrocytes (Pentikainen et al., 1979).

3.1.4 Metabolism

Metformin does not undergo metabolism in humans; however, following i.v. dosing, 20 % of dose is not recovered in urine. Therefore, some kind of transformation is suggested but no metabolites or conjugated compounds have been identified; although, hydroxylation might be a route, as in case of phenformin (Green and Feinglos, 2008).

3.1.5 Excretion

Metformin's renal clearance is > 400 mL/min, which shows that Metformin is cleared by glomerular filtration and tubular secretion.

Maximum plasma concentration time is approximately 2 hours. The plasma half-life, ranges between 4 and 6 hours (Green and Feinglos, 2008; Zhong et al., 2005).

3.1.6 Mechanism of action

Metformin reduces blood glucose concentrations by means of two mechanisms; the first one reduces glucose output by liver and the second one increases insulin effects on muscle and fat. At a molecular level, these actions are partially mediated by AMP activated protein kinase (AMP kinase) cellular activation. Mechanisms by means Metformin reduces glucose concentrations are controversial; but most of the data indicate that it reduces gluconeogenesis.

Another mechanism is reduction of glucose absorption from gut, but it does not seem to have clinical significance (Goodarzi closet al., 2005; Hundal and Inzuchi, 2003; Ristic et al., 2007).

3.1.7 Pharmacological Properties

Throughout Metformin's chronic treatment, internal absorption of vitamin B12, folates and calcium supplements is reduced, reverting then Metformin's effects on vitamin B12. Maximum effective dose is 2.5 g a day.

Metformin reduces A1c hemoglobin approximately by 2 %, much the same as sulphonylureas. It does not promote weight gain and reduces triglycerides concentrations between 15 % and 20 %. There is a consensus about reduction of microvascular complications by A1c hemoglobin reduction with any therapy (insulin or oral agents); however, only Metformin has shown to reduce cardiovascular events in Type 2 Diabetes Mellitus (Turner et al., 1999; Galeone et al., 1998).

3.1.8 Adverse reactions

Following adverse reactions may occur with Metformin treatment: taste impairment, nausea, vomiting, diarrhea, anorexia, abdominal pain and loss of appetite (CCSI V7 for metformin hydrochloride).

Gastrointestinal disorders

Gastrointestinal disorders such as nausea, vomiting, diarrhoea, abdominal pain and loss of appetite. These undesirable effects occur most frequently during initiation of therapy and resolve spontaneously in most cases. A slow increase of the dose may also improve gastrointestinal tolerability.

Skin and subcutaneous tissue disorders

Skin reactions such as erythema, pruritus, or urticaria.

Metabolism and nutrition disorders

Lactic acidosis

Decrease of vitamin B12 absorption with decrease of serum levels during long-term use of metformin. Consideration of such etiology is recommended if a patient presents with megaloblastic anemia.

Hepatobiliary disorders

Liver function tests abnormalities or hepatitis resolving upon metformin discontinuation.

Metformin is classified as category "B", for use during pregnancy and breastfeeding according to the FDA (FDA, 2015).

3.2 Gliclazide's Pharmacology

3.2.1 Gliclazide's Chemical Structure

Systematic name according to the IUPAC for Gliclazide is 1-(3, 3a, 4, 5, 6, 6a-hexahydro-1H-cyclopenta[c]pyrrol-2-yl)-3-(4-methylphenyl) sulphonylurea.

Gliclazide's empiric formula is $C_{15}H_{21}N_3O_3S$. Gliclazide's molecular weight is 323.41 g/mol (Pubchem, 2015).

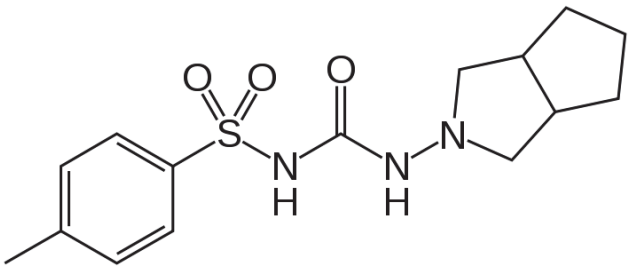

Figure 2. - Gliclazide

3.2.2 Absorption

It is quickly and well absorbed, following Gliclazide's oral dosing, plasma concentrations increase progressively until the 6th hour, and it will get steady until 6th and 12 hour. (PI Diamicron MR, 2013). Gliclazide it is fully absorbed. Intra-individual variability is low. It has a bioavailability of 80 % (Sarkar et al., 2011; Campbell et al., 1991).

3.2.3 Distribution

Gliclazide is distributed in the extracellular fluid, which causes high concentrations in the liver, kidneys, skin, lungs, musculoskeletal, intestines, and cardiac tissue when given to animals. Significant absorption in the central nervous system is unlikely. Gliclazide also crosses placental barrier and travels in the fetal blood stream. A low apparent distribution volume is likely seen in the high binding degree to proteins for Gliclazide (approximately 94 % of a plasma concentration of 8 µg/mL) (Sarkar et al., 2011).

3.2.4 Metabolism

It is metabolized widely in the liver. Less than 1 % of given oral dose appears unchanged in urine.

Metabolites are oxidative and hydroxylated byproducts, as well as glucuronic acid conjugates. Involved enzymes are cytochrome P450 2C9, cytochrome P450 2C19. No active metabolites have been detected in plasma (Sarkar et al., 2011).

3.2.5 Excretion

Approximately 70 % of the given dose is slowly cleared in urine, achieving its maximum within 7 to 10 hours following dosing. Metabolites are detectable in urine 120 h following dosing (Ings et al., 1986).

Fecal clearance is approximately 11 % of the administered dose (Ings et al., 1986). Gliclazide's elimination half-life ranges between 12 and 20 hours (IP Diamicron MR, 2013).

3.2.6 Mechanism of action

Gliclazide binds to the β cells (SUR1) sulphonylureas' receptor, blocking subsequently sensitive potassium channels ATP. Channels closure outcomes lead to the reduction in the potassium flow which causes β cells depolarization. This opens β cells voltage dependent calcium channels; which in turns, results in the calmodulin activation then leading insulin's exocytosis containing granules secretion (Hoich et al., 1986).

3.2.7 Pharmacological Properties

Gliclazide is a second generation sulphonylurea commonly used for the treatment of Type 2 Diabetes, used in poorly controlled patients with Metformin as monotherapy, and provides a complementary mechanism of action for Metformin (Ristic et al., 2007).

3.2.8 Adverse reactions

Overall, they depend on the administered dose; they are transient and respond to the therapy's reduction and stop.

As any other sulphonylureas, Gliclazide therapy may cause hypoglycemia.

Possible hypoglycemic symptoms are: headache, increase appetite, nausea, vomit, tiredness, drowsiness, sleep disorders, restlessness, aggressiveness, reduced concentration, wakefulness and reaction; depression, confusion; visual and speech disorders; aphasia; tremors, paresis, sensory disorders, dizziness, helplessness feeling, lack of self-control, delirium, seizures, shallow breathing, bradycardia, drowsiness, syncope which might lead to coma and death.
In addition, the following symptoms might be seen: sweating, anxiety, tachycardia, hypertension, palpitations, angina pectoris and cardiac arrhythmias.

Usually, symptoms disappear after intake of carbohydrates (sugar). However, artificial sweeteners have no effect. Experience with other sulphonylureas shows that hypoglycemia can recur even when measures prove effective initially.

Gastrointestinal disturbances, including abdominal pain, nausea, vomiting dyspepsia, diarrhea, and constipation have been reported: if these should occur they can be avoided or minimized if Gliclazide is taken with breakfast. The following undesirable effects have been more rarely reported:

• Skin and subcutaneous tissue disorders: rash, pruritus, urticaria, angioedema, erythema, maculopapular rashes, bullous reactions (such as Stevens-Johnson syndrome and toxic epidermal necrolysis).
• Blood and lymphatic system disorders: Changes in hematology are rare. They may include anemia, leucopenia, thrombocytopenia, granulocytopenia. These are in general reversible upon discontinuation of medication.
• Hepato-biliary disorders: raised hepatic enzyme levels (AST, ALT, alkaline phosphatase), hepatitis (isolated reports). Discontinue treatment if cholestatic jaundice appears. These symptoms usually disappear after discontinuation of treatment.
• Eye disorders
Transient visual disturbances may occur especially on initiation of treatment, due to changes in blood glucose
• Class attribution effects:
As for other sulphonylureas, the following adverse events have been observed: cases of erythrocytopenia, agranulocytosis, hemolytic anemia, pancytopenia, allergic vasculitis, hyponatremia, elevated liver enzyme levels and even impairment of liver function (e.g. with cholestasis and jaundice) and hepatitis which regressed after withdrawal of the sulphonylurea or led to life-threatening liver failure in isolated cases (EMC SmPC Diamicron).

Gliclazide is classified as category "C" for its use during pregnancy and breastfeeding as per the FDA (FDA, 2015).

4 Adverse Reactions of the combination product (Metformin/Gliclazide)

Hypoglycemia

As for other sulphonylureas, treatment with Gliclazide can cause hypoglycemia, if mealtimes are irregular and, in particular, if meals are skipped. Possible symptoms of hypoglycemia are: headache, intense hunger, nausea, vomiting, lassitude, sleep disorders, agitation, aggression, poor concentration, reduced awareness and slowed reactions, depression, confusion, visual and speech disorders, aphasia tremor, paresis, sensory disorders, dizziness, feeling of powerlessness, loss of self-control, delirium, convulsions, shallow respiration, bradycardia, drowsiness and loss of consciousness, possibly resulting in coma and lethal outcome.

In addition, signs of adrenergic counter-regulation may be observed: sweating, clammy skin, anxiety, tachycardia, hypertension, palpitations, angina pectoris and cardiac arrhythmia.

Usually, symptoms disappear after intake of carbohydrates (sugar). However, artificial sweeteners have no effect. Experience with other sulphonylureas shows that hypoglycemia can recur even when measures prove effective initially. If a hypoglycemic episode is severe or prolonged, and even if it is temporarily controlled by intake of sugar, immediate medical treatment or even hospitalization is required.

Blood and lymphatic system disorders

Rare: Hematologic disturbances which may include anemia, leucopenia, thrombocytopenia, granulocytopenia. These are in general reversible upon discontinuation of medication.

Metabolism and nutrition disorders

Very rare: Lactic acidosis. Decrease of vitamin B12 absorption with decrease of serum levels during long-term use of Gliclazide. Consideration of such etiology is recommended if a patient presents with megaloblastic anemia.

Frequency not known: Hypoglycemia.

Nervous system disorders

Common: Taste disturbance.

Eye disorders Frequency not known: Transient visual disturbances may occur especially on initiation of treatment, due to changes in blood glucose levels. Gastrointestinal disorders

Very common: Gastrointestinal disorders such as nausea, vomiting, diarrhea, abdominal pain, and loss of appetite.

Frequency not known: Constipation and dyspepsia.

These undesirable effects can be avoided or minimized if dose is increased slowly and Gliclazide is taken with breakfast.

Hepatobiliary disorders

Very rare: Liver function tests abnormalities or hepatitis resolving upon treatment discontinuation.

Discontinue treatment if cholestatic jaundice appears.

Skin and subcutaneous tissue disorders

Very rare: Skin reactions such as erythema, pruritus, or urticarial.

Frequency not known: Angioedema, maculopapular rashes, and bullous reactions (such as Stevens-Johnson syndrome and toxic epidermal necrolysis).

Related to sulphonylureas

Class attribution effects:

As one of the active ingredients in Gliclazide belongs to sulphonylureas, the following adverse events have been observed: cases of erythrocytopenia, agranulocytosis, hemolytic anemia, pancytopenia, allergic vasculitis, hyponatremia.

5 Study Objectives

5.1 Primary Objective

To assess the food effect on bioavailability ($AUC_{0-\infty}$, AUC_{0-t} and C_{max}) for the fixed dose combination Metformin 1000 mg/Gliclazide 30 mg MR, given as single dose in fed and fasted state to healthy volunteers.

5.2 Secondary objectives:

- Additional Pharmacokinetic parameters will be determined: t ½, t_{max}, Vz/f and Cl/f.
- To assess and compare safety and tolerability of the fixed dose combination Metformin /Gliclazide administered in fast and fed state.

6 Investigational Plan

6.1 Experimental design and study plan

The IMP will be given in fast and fed state. A randomized, open label, single dose, 2 x 2 cross design will be used with a 14-day washout period between 2 study stages. Treatment groups will be balanced, with the same number of subjects, who will be randomized to the dosing sequences (see table below).

Food's effect study			
Sequence	**Period 1**	**Washout period**	**Period 2**
1	Fast		Fed
2	Fed		Fast

All data from participant subjects should be analyzed and included in the statistical analysis, as long as they meet the established criteria.

6.2 Rationale for Study Design

As per international clinical guidelines about Type 2 Diabetes management (Diabetes Care, 2014), Metformin, if there are no contraindications and if this is tolerated, is the drug of choice for Type 2 Diabetes. In the event Metformin monotherapy at the maximum tolerated dose does not maintain A1c target for more than 3 months, then it will be necessary to add a second oral agent from another pharmacological family, such sulphonylureas, thiazolidine, DPP4i or GLP-1-RA.

Gliclazide is a second generation sulphonylurea which offers a complementary mechanism of action for Metformin. On the other hand, there is wide experience about Metformin and Gliclazide association and it is particularly recommended by some clinical guidelines (Theobald, 2014) when Metformin monotherapy is not enough to achieve treatment objectives.

Therefore, a fixed dose combination has been developed as second line therapy, in cases when Metformin's monotherapy has not shown a satisfactory benefit/risk ratio. Therefore, this fixed dose combination for Metformin and Gliclazide is a generic for the existent concomitant administration of these two active ingredients which use is already generalized. Fixed dose combination has the potential advantage of making easier the therapy by reducing the number of individual dosage units to be taken by the patient. This simplifies therapy and may improve patient's compliance (Diabetes Care, 2014; Treatment Algorithm for Type 2 Diabetes, 2014.)

Prior to this proposed study, a study to assess bioequivalence between fixed dose and concomitant administration was performed, in addition to the lack of drug - drug interaction.

According to the international guidelines, studies to compare relative bioavailability in fasting and food conditions are necessary for combined modified release drugs, such as the combination proposed herein. Therefore, as part of the full clinical development for the fixed dose combination, food interaction should be studied. Study design adheres to the Mexican regulations as well as to the international guidelines for food assessment (Diabetes Care, 2014).

The purpose of this clinical study is to assess the food effect on bioavailability for the new fixed dose combination for Metformin 1000 mg/Gliclazide 30 mg MR.

In addition to the inclusion and exclusion criteria, potentially confounding factors to be controlled are: fast state and dosing immediately after food intake, as well as the type breakfast; morning schedule for treatment's dosing; volume and type of liquids to drug's intake; schedule, type and amount of food; physical activity restricted to minimum which does not modify heart rate; supine or seated positions up to reaching the stated time for t_{max}; exclusion of caffeine and/or theophylline products; controlled access to the health services until reaching stated time for t_{max}. In addition, concomitant use of drugs is not allowed, unless prescribed drugs for adverse events, with the exception for paracetamol that can be given if required, as per the Principal Investigator's judgment

This study will be carried out in male and female healthy volunteers.

6.3 Study population screening

Only those people who meet all inclusion criteria and no exclusion criteria will be recruited as study subjects. Before performing any study specific assessment which is not part of the subject's routine health care, the Principal Investigator will make sure that the subject has provided his/her written consent form according to the procedure described in section 9.2, Subject's information and informed consent form.

- Recruitment by invitation by means of an open announcement.
- Inclusion of 22 subjects in study as per protocol's criteria.
- Female and male (at least 40 % for each gender by group).

6.3.1 Inclusion criteria:

Subject grants informed consent prior any study related activity will be performed.

1. Ethnicity: Mexicans (e.g., Caucasian, native people and Mestizo [mixed race])
2. Gender: Female and male (at least 40 % for each gender by study group).
3. Age between 18 and 55 years old, both including.
4. Weight between 55 and 95 kg.
5. Body mass index between 18 and 27 kg/m^2.
6. Non smokers or subjects who do not smoke more than 5 cigarettes or 1 pipe a day
7. Good physical and mental health based on the clinical history and physical examination.
8. All results from blood chemistry, hematology, and urinalysis should be within normal ranges or without clinically significant deviations as per Principal Investigator's judgment*.
9. Hematology complete blood count [CBC]: hematocrit and hemoglobin must be above the lower limit; upper limit may range up to 15 %. Remaining results, including white blood cells may range ± 15 %, if subject is asymptomatic.
10. Liver Function Test, including direct and indirect bilirubin, glutamic oxaloacetic transaminase (aspartate aminotransferase) and glutamic pyruvic transaminase (alanine aminotransferase), total proteins, albumin, globulin and albumin/globulin ratio. Total bilirubin from 0.00 to 1.6 mg/dL, direct bilirubin from 0.0 to 0.4 mg/dL and indirect bilirubin from 0.0 to 1.2 mg/dL. They may range up to 15 % the enzyme's upper limit. Lower limit has no restriction for subjects' inclusion.
11. Electrocardiogram (12 leads) without clinically significant pathological signs, particular in QTc interval Bazett (normal value < 450 ms).

12. All women of childbearing potential should test negative for pregnancy qualitative and quantitative test during screening period and at the start of each visit (day -1 for each treatment period.)

13. Vital signs (blood pressure and pulse) in supine position within normal ranges or with clinically significant abnormalities as per the Principal Investigator's judgment.
    f. Heart rate between 50 and 100 beats per minute.
    g. Respiratory rate between 12 and 20 per minute.
    h. Systolic blood pressure between 80 and 129 mmHg.
    i. Diastolic blood pressure between 50 and 89 mmHg.
    j. Forehead temperature between 36.0 and 37.0 °C.

14. All women of childbearing potential who are not pregnant or breastfeeding and who are using a highly effective contraceptive method (defined as those, alone or combined, which failure rate is low; i.e., less than 1 % a year, when used continuously and correctly) for at least one month before and following dosing. Barrier methods and intrauterine device are considered standard contraceptive methods. Hormonal methods will not be included. Post-menopausal women can be included (i.e., those with at least 12 consecutive months of amenorrhea following their last menstruation period) or surgically sterile/hysterectomy for at least 6 months before their participation in the study.

15. Negative result for alcohol breath test and urine test for drugs of abuse (opioids, barbiturates, cocaine and its metabolites, amphetamines, metamphetamines, morphine, cannabinoids, benzodiazepines and tricyclic antidepressants), at screening and at each day -1 of the 2 treatment periods.

16. Negative serology tests for human immunodeficiency virus (HIV1 and HIV2 antibodies), hepatitis A (HAV), hepatitis B (HBV), hepatitis C (HCV) and VDRL test screening.

6.3.2 Exclusion criteria:

1 Subjects who have received any investigational drug within 90 days prior to the study start.

2 Subjects who have donated or lost 450 mL or more of blood within 90 days prior to the study start.

3 Subjects with history of cardiovascular, renal, liver, metabolic, gastrointestinal, neurological, endocrine, or hematopoietic (any type of anemia) diseases; mental disease, surgery or other organic abnormalities which might affect the study of the investigational drug pharmacokinetics.

4 History of gastrointestinal tract surgery that might affect gastrointestinal absorption and/or motility as per the investigator's judgment.

5 Subjects with history of hypersensitivity to the study drug and/or any formulation's ingredient; history of drug induced anaphylaxis.

6 Subjects who take any other drug, prescribed or over the counter drugs 30 days before the study drug dose and for which at least seven elimination half-lives had not elapsed, including multi-vitamins and herbal products (e.g., St. John's Wort) including ASA and oral contraceptives for women. Except for paracetamol that can be given if required, as per the Principal Investigator's judgment.

7 Renal failure or renal impairment (creatinine clearance < 80 mL/min), assessed by using the Cockcroft-Gault formula.

8 Subject's disagreement or lack of capacity to communicate and cooperate with the Investigator, lack of legal capacity or limited legal capacity which prevent him/her from continuing in the study.

9 Refusal of high-fat diet which is necessary to assess the food effect. Considerable deviations to the diet's normal nutritional patterns.

10 Subjects who have smoked tobacco, having drunk alcohol, or xanthines containing beverages or food (coffee, tea, cacao, chocolate, mate, coke drinks, etc.) above 600 mg of caffeine a day (a 240-mL cup of coffee contains 100 mg of caffeine, one cup of tea contains 30 mg and a glass of coke contains 20 mg of caffeine); those who have had grilled food (charcoal) within 24 h prior to the drug dosing (*Fuhr 1998*).

11 Intake of grapefruit, orange, cranberries or their juices within 14 days prior to the drug's dosing and throughout the study.

12 Legal inability or limited legal capacity

13 Incarcerated subjects.

14 Subjects who have been exposed to agents known as liver enzyme systems' inducers or inhibitors, or who have taken potentially toxic drugs within 30 days prior to the study.

6.3.3 Study withdrawal criteria

Subjects can be withdrawn from the study, as per the Principal Investigator's judgment, for the following reasons:

1. In case continuous participation in the trial is detrimental for subject's health, as per Investigator's judgment.

2. Hypertension signs and symptoms which require pharmacological treatment.

3. Syncope or shock.

4. Severe adverse reaction.

5. Serious Adverse Event.

6. Concurrent diseases or concomitant treatments not allowed by the protocol.

7. Lack of discipline.

8. Lack of compliance with the diet.

9. Loss of two samples within absorption period or near to the C_{max}.

10. Should emesis occur within the established time for t_{max}.

11. Data from subjects who experience emesis during the course of the bioavailability study should be removed from the statistical analysis if vomiting occurs in or after twice the median value for t_{max}.

12. If any of the participant subject, during wash-out period, takes alcoholic beverages, smokes tobacco, has xanthines containing beverages or food (coffee, tea, cacao, chocolate, mate, coke drinks, etc.) grapefruit, grapes or citrus, or grilled food (charcoal) within 24 h prior to the recruitment in the admission phase, or using any drug known by affecting the study.

13. Positive test for drugs of abuse (drug screening) such as: amphetamines, benzodiazepines, cocaine, metamphetamines, morphine and tetrahydrocannabinoids.

14. Positive pregnancy test, qualitative and quantitative test during screening period and at the start of each visit (day -1 for each treatment period) following the study drug dosing.

15. All cases where the Principal Investigator judges as lack of compliance to the protocol or which may risk final results.

16. Lost to follow-up.

17. Participation in another investigational study.

18. Any event which in the opinion of the Investigator might risk subject's safety unacceptably.

6.4 Start-up criteria for the study treatment

- All women of childbearing potential should test negative for pregnancy qualitative and quantitative test at day -1 of each treatment period.
- All subjects should have negative results for alcohol screening (breath test) at day -1 for each treatment period and drugs of abuse (opioids, barbiturates, cocaine and its metabolites, amphetamines, metamphetamines, morphine, cannabinoids, benzodiazepines and tricyclic antidepressants) on each admission (day -1 for each treatment period).

6.5 Study Subject Withdrawal

Participant subjects in the study are free to drop the study at any moment and they will only be asked to give the reason of their drop out.

Drop out reason should be documented in the medical chart and in the case report form. Study drug assigned to the dropped subject cannot be assigned to another subject. Subjects who drop out will not be replaced.

6.5.1 Early study termination

The clinical study can be finished or stopped as per health authorities' request, in case of new safety or efficacy information posing a unfavorable risk - benefit for study subjects.

The Sponsor may end the study in case it is no longer justified from the medical or ethical point of view; due to poor enrollment, due to the stop of the study drug's clinical development, withdrawal of the study drug or comparator from the market due to safety reasons. Health Authorities, Independent Ethics Committee (IEC) and Investigational Committee (IC) will be informed about this cancellation as per the applicable regulations.

- This study can be stopped temporarily or definitively as per the Sponsor, Principal Investigator, or Ethics and Investigational Committee according the Ministry of Health's guidelines.
- Reasons can be several, among others, frequency and type of adverse events which lead to a considerable number of subjects to abandon the study; new information about the drug which affect directly subjects' safety or study conduct; or based on the Ethics and Investigational Committee, Investigational Committee and Ministry of Health's decision.
- In case of temporal or early termination, research subjects will be immediately informed and an appointment will be scheduled to let them know the definitive decision about the study course. In case of definitive termination, the Principal Investigator will inform the study subjects about this decision and will determine the withdrawal procedures to be performed.
- If termination is due to the Sponsor or Principal Investigator's decision, decision between both of them should be documented in writing, providing the Ethics and Investigational Committee, the Investigational Committee and Ministry of Health with a detailed explanation about the temporary or definitive termination.
- Study drugs will be secured and kept, and will be disposed (return or storage) as agreed with the Sponsor.

6.5.2 End of Study Definition

The end of the trial is defined by the last contact with the last subject who participated in the trial (last subject last visit).

7 Investigational Drug

7.1 Investigational Drug Description

Study Sponsor will provide drugs to be given to the subjects in each of the study visits.

- International non-proprietary name: Metformin/Gliclazide.

- Test drug: Metformin 1000 mg/Gliclazide 30 mg MR.
- Dosage form: tablets
- Quali-Quantitative Formula: Each tablet contains Metformin 1000 mg/Gliclazide 30 mg MR.
- Manufacturer: Merck, SA, Brazil.
- Distributor: Merck, SA, Brazil.
- Marketing Holder: Merck, SA, Brazil.
- Dosage: Metformin 1000 mg/Gliclazide 30 mg (one tablet)

Test drug should be imported as per the following quantities and specifications:

Description: Fixed combination tablet of Metformin 1000 mg/Gliclazide 30 mg.

Batch number:

Laboratory's Approval Number

Quantity required for each stage of the research: 12 tablets.

44 tablets for the study.

44 tablets as retention sample.

Manufacturer:

Merck, SA, Brazil.

Product's importer:

PPD

Ph. PPD
Tax ID number: PPD :

7.2 Dosage and administration

The IMP (investigational medicinal product) will be given fast or fed state orally at a dose of Metformin 1000 mg/Gliclazide 30 mg (one tablet) with 250 mL of water. Only a single dose will be administered.

Fast treatment: subjects should fast for at least 10 hours before dosing. IMP will be dosed orally with 250 mL of water. No food intake is allowed for 4 hours following drug's dosing. Subjects be allowed water ad libidum except for 1 hour before and after drug administration.

Fed treatment: following night fasting for at least 10 hours, subjects should be provided the recommended food 30 minutes prior to the IMP dosing. They will be given 30 minutes as maximum for finalizing the food intake. IMP will be given 30 minutes following the start of food intake. Drug should be given with 250 mL of water. No food intake is allowed for 4 hours following drug's dosing. Subjects be allowed water as desired except for 1 hour before and after drug administration.

7.3 Randomization to treatment groups

Once it is confirmed that all inclusion criteria and none exclusion criteria have been met, a consecutive number in ascendant order will be assigned to subjects, starting by 1 up to the last case (22). For each one of these numbers, it will correspond one of the sequences R (fasted conditions) - T' (fed conditions) or T' (fed conditions) - R (fasted conditions) from a randomization list, previously generated by the site www.randomization.com and in accordance with the standard operating procedure code: PPD , RANDOMIZATION IN AN INTERCHANGEABILITY AND BIOCOMPARABILITY CLINICAL STUDY

7.4 Selection and Dosing Schedule for Each Subject

Twenty two male and female subjects will be included in the study. Both genders will be included in a minimum ratio of 40 % at least for each gender by study group, it is intended to include an equal number of females and males.

The IMP dosing will occur in the morning (around 8 a.m.) following fasting (at least 10 hours) and according to the randomization list. Drug's dosing will be documented in the case report form.

The IMP will be dosed for all subjects from 08:00 till 8:10 h, in 5 groups of 4 subjects and 1 of 2, with a 2-minute gap among each group for dosing and each one of the activities. Groups' distribution can be changed due to operational reasons, as long as there is no impact in the study results.

Groups' distribution, subjects and dose schedules are shown in the following table.

Dosing scheme

Group	No. of subjects	Dosing schedule
A	01, 02, 03 and 04	08:00 h
B	05, 06, 07 and 08	08:02 h
C	09, 10, 11 and 12	08:04 h
D	13, 14, 15 and 16	08:06 h
E	17, 18, 19 and 20	08:08 h
F	21 and 22	08:10 h

7.5 Concomitant therapies and drugs

Subjects who take any other drug, (prescribed or over the counter drugs) within 30 days before the first IMP administration and for which at least seven elimination half-lives had not elapsed, are not eligible. This includes e.g. multi-vitamins and herbal products (e.g., St. John's Wort) as well as ASA and oral contraceptives for women. The only exception is given for paracetamol which may be given if required, as per the Principal Investigator's judgment (see section 7.5.1)

All concomitant drugs taken by the subjects during the study, starting as per informed consent signature have to be appropriately recorded in the Case Report Form (CRF), stating the name, dose and indication for each drug. Non-pharmacological interventions or any other intervention should be recorded in the CRF.

7.5.1 Allowed Drugs

Concomitant use of drugs is not allowed, unless those prescribed for adverse events (except for paracetamol [acetaminophen], which can be given if necessary) and to protect subjects' well-being who will be allowed to continue in the study as per the Principal Investigator's judgment. This should be justified in the medical chart. As long as the drug does not interfere with the study medication.

Occasional use of paracetamol will be permitted within the screening period. The investigator may allow the subject to take paracetamol in case of pain during the trial, but the dose should not exceed 1000 mg/day. The administration of paracetamol is limited to 3 consecutive days per period. The amount of paracetamol administered must be recorded in the CRF.

7.5.2 Forbidden Drugs

Subjects who take any other drug, prescribed or over the counter drugs 30 days before the first IMP administration and for which at least seven elimination half-lives had not elapsed, including multi-vitamins and herbal products (e.g., St. John's Wort) in addition to acetylsalicylic acid and oral contraceptives for women (with paracetamol being the only exemption). Other Interventions

Subjects who have been exposed to agents known as inducers or inhibitors of liver enzymatic systems, who have taken drugs potentially toxic within 30 days prior to the study start-up, who require any drug throughout the study, who have history of gastrointestinal tract surgery which might affect gastrointestinal absorption and/or motility are not allowed in the study or to continue in the study as per the Principal Investigator's judgment.

7.5.3 Special Warnings

In case of adverse events, medical staff will provide medical care required depending on the adverse event occurred. In case of severe eventuality, which requires hospital admission, the Principal Investigator or medical staff on due will request the transfer of the subject to the agreed hospital and will contact the external ambulance service, according to the standard operating procedure, code: PPD , MANAGEMENT OF CLINICAL EVENTUALITIES.

7.5.4 Management of Specific Adverse Events or Adverse Drug Reactions

In case of adverse events, medical staff will provide immediate medical care required depending on the adverse event occurred. The Study Principal Investigator will decide care following to the event.

7.6 Investigational Drug Packaging and Labeling

The IMP (combination of fixed dose of Metformin 1000 mg/Gliclazide 30 mg MR) will be provided by the Sponsor, in its primary and secondary original packages or prototype.

Drug will be stored in a closed package, identified with an external label containing study code and, if applicable, an internal label containing the study code, batch number and expiry date as minimum. Drug will be stored according to the products' needs.

All study drugs will be packaged and labeled according to the applicable standards and directives from Good Manufacturing Practices.

7.7 Preparation, Management and Storage of Study Drug

The IMP will be received at the PPD by the Samples Processing and Pharmacy Coordinator or by the designated staff, as per the standard operating procedures, code: PPD : *RECEIPT, USE, BALANCE AND*

FINAL DISPOSITION OF THE STUDY DRUG. Once drugs have been identified, they will be kept by the clinical unit they are administered to the subjects.

Acceptance criteria for drugs.

- Complete documentation:

✓ Copy of the certificate of analysis which contains quality control tests, including:

  - Assessment.
  - Uniformity of expressed dose as content uniformity, if applicable.
  - Dissolution.

✓ Copy of the letter issued by the Sponsor, specifying that the drug meets with Good Manufacturing Practices for drug.

- Test drug with label compliant with the minimum requirements from NOM-072-SSA1-2012, Labeling of Drugs and Herbal Remedies.
- Enough quantities of the study drug to conduct the study and keep some as "retention samples".
- Expiry date not due at the moment of use in the clinical study.
- Test and reference drug in good physical conditions.

Rejection criteria for IMP.

- Blisters which have been tampered.
- Drug received out of the storage or transportation specifications.
- In case primary package is damaged.

Drug should be stored in a safe, dry, and locked cabinet at a temperature not higher than 25 °C or 30 °C according to the label information.

7.8 Study Drug Accountability

The Sponsor will provide enough quantities of the IMP (investigational medicinal product) to conduct the study to the PPD and keep "retention samples" as requested by the authority. The Principal Investigator, Sample Processing and Pharmacy Coordinator or designated staff at the PPD will be in charge of dispensing the number of required doses the day of the study as well as of keeping an inventory of used and stored doses as retention samples, according to the standard operating procedure, code: PPD : RECEIPT, USE, BALANCE AND FINAL DISPOSITION OF THE STUDY DRUG.

7.9 Assessment of Study Drug Compliance

Throughout dosing period, dose, full intake and time will be checked and recorded in the appropriate forms, according to the standard operating procedure, code: PPD PREPARATION AND ADMINISTRATION OF DRUGS IN INTERCHANGEABILITY AND BIOCOMPARABILITY CLINICAL STUDIES.

A subject is compliant with the treatment only if he/she completes both study periods.

7.10 Blinding

Randomization codes for dosing sequence will be kept by the Principal Investigator. The analytical unit, in charge of the biological samples processing will be blinded throughout the samples analysis, according to the standard operating procedures.

7.11 Overdose treatment

Overdose is defined as any dose higher than the highest daily dose included in the clinical trial protocol or foreseen for a subject recruited in the study. Even though this does not meet criteria for serious adverse event, any overdose should be recorded in the study drug section of the CRF and medical chart. It should be reported to the National Center of Pharmacovigilance (CNFV) from the Federal Commission for the Protection against Sanitary Risks (COFEPRIS), according to the standard operating procedures, code: PPD , ADVERSE EVENT REPORTING IN CLINICAL AND BIOAVAILABILITY STUDIES and the Mexican Official Standard for Setting-up and Operations of Pharmacovigilance in Mexico, NOM-220-SA1-2012 and following procedure listed in section 7.5.1.

In this trial, the IMP will be administered under medical supervision as 2 oral single doses of one tablet of the fixed dose combination of 1000 mg Metformin and 30 mg Gliclazide separated by a 14-day washout period.

Overdosage is accordingly unlikely, however in case of overdose, close monitoring will be performed and adequate medical care will be provided based on the clinical judgment of the Investigator (for further details see text below).

Gliclazide overdose:

Study drug overdose may lead hypoglycemia.

Moderate symptoms of hypoglycemia without unconsciousness or neurological sign should be fully corrected by means of glucose administration. Observation and strict control should continue until the Principal Investigator is sure that the subject is not at risk anymore.

Severe hypoglycemic reactions such as coma, seizures and other neurological disorders are possible and they should be managed as a medical emergency which requires immediate hospital admission.

During the study all subjects will have their finger stick blood glucose measured (see section 8.5) and will be will be applied intravenously 10 mL of concentrated glucose solution 10 % within 1 or 2 hours following the dose. Glucose solution charges are not limited to these two occasions. Should hypoglycemia symptoms occur, additional glucose solution can be given. The concentrated glucose solution 10 % should be given slowly, preferably through a small bore needle into a large vein, to minimize venous irritation.

Subject should be closely controlled, based on his condition following that episode and the Principal Investigator will make a decision whether subsequent control is necessary.

Metformin overdose:

Hypoglycemia has not been seen with metformin doses of up to 85 g, although lactic acidosis has occurred in such circumstances.

High overdose of metformin or concomitant risks may lead to lactic acidosis. Lactic acidosis is a medical emergency and must be treated in hospital. The most effective method to remove lactate and metformin is hemodialysis.

Symptoms and Management of overdose of the combination drug product (metformin & Gliclazide)

Symptoms

No data are available with regard to overdose of the fixed-dose combination product.

Symptoms of a metformin overdose

High overdose of metformin or concomitant risks may lead to lactic acidosis. Lactic acidosis is a medical emergency and must be treated in hospital.

Symptoms of a Gliclazide overdose

An overdose of sulphonylureas may cause hypoglycemia.

Management

Management of a metformin overdose

The most effective method to remove lactate and metformin is hemodialysis.

Management of a Gliclazide overdose

Moderate symptoms of hypoglycemia, without any loss of consciousness or neurological signs, must be corrected by carbohydrate intake, dose adjustment and/or change of diet.

If hypoglycemic coma is diagnosed or suspected, the patient should be given a rapid i.v. injection of 50 mL of concentrated glucose solution 30 %. This should be followed by continuous infusion of a more dilute glucose solution (10 %) at a rate that will maintain blood glucose levels above 1 g/L. Patients should be monitored closely and, depending on the patient's condition after this time, the doctor will decide if further monitoring is necessary.

Gliclazide is not dialyzable due to its strong binding to proteins.

7.12 Medical Care for Subjects Following the End of the Study

External follow-up will be given to all subjects recruited in the study. As per the last dose of the drug, free appointment or telephone contact will be available as per the Principal Investigator's judgment, for a period equivalent to the established wash-out period.

8 Study Assessments and Procedures

8.1.1 Subjects screening

Once the Informed Consent Form has been signed, subject's eligibility will be checked according to the inclusion and exclusion criteria, including the result of all investigations. Preparation and update of clinical chart will be made according to the Standard Operating Procedure, code: PP , PREPARATION AND HANDLING OF MEDICAL CHART DIN INTERCHANGEABILITY AND BIOCOMPARABILITY STUDIES, at the PPD In the event the subject already has a medical chart, clinical history will only be updated.

Subjects should have:

- Laboratory tests within 21 days prior to the start of the study.
- Electrocardiogram within 21 days prior to the start of the study, unless any pathology has been reported during that period of time.

8.1.2 Study periods

At the beginning of each period, a clinical assessment consisting of an examination by apparatus and systems by means of a physical examination will be made in order to check if the subject remains eligible for the study. Blood sample will be taken for pregnancy tests for women of childbearing potential and urine sample for drug screening. Alcohol breath test will also be performed.

Subjects should inform the medical staff in charge of the study conduct about any symptom they might have. Likewise, medical staff will question the subjects in every study period about symptoms occurred since recruitment in the first period, prior and following dosing. In case they report any, they will be provided with assistance and notes will be made in the appropriate documents.

During admission, subjects will be continuously monitored and an electrocardiogram will be performed pre-dose, during discharge (48 h post-dose) and during the last outpatient sampling (168 h post-dose) at each visit.

During each admission, blood and urine samples will be taken to subjects for safety laboratory tests such as hematology, blood chemistry - 6 elements (glucose, urea, creatinine, uric acid, cholesterol and triglycerides), prothrombin time (PT) and partial thromboplastin time (PTT) and urinalysis. Samples will be taken pre-dose (following a 10-h fasting period) and at discharge (48 h post-dose).

Glucose measurements will be performed: pre-dose and 2, 4, 12, 24, and 32 h post-dose. Finger stick glucose measurement (Hematoglucotest) will be made pre-dose and 2, 4, 8, 12 and 24 h post-dose.

At the end of each period, a clinical assessment consisting of an interview, physical examination (including general appearance, skin, head, neck (including thyroid), ears, nose, throat, cardiovascular and pulmonary system, abdomen, neurological, peripheral vascular, and musculoskeletal system oral cavity examination), vital signs measurement and follow-up of adverse events will be made. Blood and urine samples will be taken for safety laboratory tests such as hematology; blood chemistry - 6 elements (glucose, urea, creatinine, uric acid, cholesterol and triglycerides), prothrombin time (PT) and partial thromboplastin time (PTT); and urinalysis.

At the last visit for outpatient sampling (168.00 h post-dose) a clinical assessment consisting of an interview, physical examination (see above) will be performed. Weight and height, and vital signs will be measured; and electrocardiogram and adverse events follow-up will carried out. Blood sample will be taken for pregnancy tests for women of childbearing potential. For post-menopausal women (amenorrhoeic for at least 12 months), post-menopausal condition will be confirmed by means of follicle-stimulating hormone level measurement during screening period only.

Due to operational issues, the Principal Investigator may modify start of the study (starting as per the dosing of study drug) for one hour, as long as there is no impact on the study results.

8.1.3 Control of Meals and Liquid Intake

Subjects from **R** treatment should fast for at least 10.5 hours before the dosing and at least for 4 hours following the dosing. The study drug will be administered (08:00 h). Water is allowed *ad libitum*, except for the period between one hour prior to dosing and the first meal.

Subjects from the **T'** treatment will have a standardized breakfast after a fast of at least 10.5 hours. Study subjects have to eat this meal in 30 minutes. 30 minutes after start of the breakfast the study drug will be administered (08:00 h). Water is allowed *ad libitum* 1 hours following the dose.

During the rest of confinement, meals will be served as shown below. Forty five minutes (45) will be allowed for having meals; water consumption will not be subject to this period.

R' treatment's meals schedule

MEALS	DAYS 0 and 14,	DAYS 1 and 15	DAYS 2 and 16
Breakfast	-----	As from 07:30 h	As from 09:00 h
Lunch	-----	As from 16:00 h	As from 15:00 h
Dinner	21:00 h	As from 21:00 h	As from 20:00 h

T treatment's meals schedule

MEALS	DAYS 0 and 14,	DAYS 1 and 15	DAYS 2 and 16,
Breakfast	-----	As from 12:00 h	As from 09:00 h
Lunch	-----	As from 16:00 h	As from 15:00 h
Dinner	21:00 h	As from 21:00 h	As from 20:00 h

Groups will be unsynchronized for 2 minutes among them for food delivery which is appropriate for the dosing order and interval.

Study breakfast consist of two fried eggs with butter, two bacon strips, two toasts with butter, 113 g of potato croquettes and 240 mL of whole milk (approximately 800 to 1000 calories) (150, 250, and 500-600 calories from protein, carbohydrate, and fat, respectively), substitutions in this test meal can be made as long as the meal provides a similar amount of calories from protein, carbohydrate, and fat and has comparable meal volume and viscosity. (FDA, 2002, EMA 2013).

8.2 Schedule of Assessments

Prior any study assessment, the Principal Investigator or designated person will obtain written informed consent from the study subjects.

8.2.1 First Administration Period

- Admission the day prior to the visit for assuring 10.5-h fasting period.
- Dinner at least 10.5 h before the dosing in fasted condition or breakfast in fed condition.
- Subject's eligibility will be checked again before catheter insertion and drug's dosing.
- Catheter insertion at the moment of blood sampling for gonadotropins determination (elective, only for women).

First day (treatment R):

- At 5:00 h, grooming and intake of 250 mL of water.
- Between 05:20 and 06:30 h electrocardiogram.
- Following electrocardiogram the following activities will be performed:

✓ Vital signs measurement.

✓ Catheter insertion (control blood sampling).

✓ Blood sampling for safety laboratory tests.

✓ Finger stick for blood glucose determination.

✓ Adverse events and prior medication.

✓ Urine sampling for safety laboratory tests.

- At 08:00 h, oral administration of IMP with 250 mL of water at room temperature p.o., according to the standard operating procedure, code: PPD : PREPARATION AND ADMINISTRATION OF DRUGS IN INTERCHANGEABILITY AND BIOCOMPARABILITY CLINICAL STUDIES.
- From 08:30 to 00:00 h, sampling as per the schedule of assessments.
- At 09:00 h, intravenous administration of 10 mL of 10% glucose solution.
- At 10:00 h Measurement of blood glucose and capillary glucose on a test strip (hemoglucotest), 2 hours post dose and intravenous administration of 10 mL of 10% glucose solution.
- Finger stick and IV blood glucose, vital signs measurement 4 hours following dose.
- As from 12:00 h, breakfast.
- Finger stick for blood glucose determination and vital signs 8 hours following dose.
- As from 16:00 h, lunch.
- Finger stick and IV blood glucose, vital signs measurement 12 hours following dose.
- As from 21:00 h, dinner.

First day (treatment T': food + drug):

- At 5:00 h, grooming and intake of 250 mL of water.
- Between 05:20 and 06:30 h electrocardiogram.
- Following electrocardiogram the following activities will be performed:
- ✓ Vital signs measurement.
- ✓ Catheter insertion (control blood sampling).
- ✓ Blood sampling for safety laboratory tests.
- ✓ Finger stick for blood glucose determination.
- ✓ Adverse events and prior medication.
- ✓ Urine sampling for safety laboratory tests.
- At 7:30 h, breakfast. They will be given 30 minutes as maximum for food intake (FDA, 2002).
- At 08:00 h, oral administration of IMP with 250 mL of water at room temperature p.o., according to the standard operating procedure, code: PPD : PREPARATION AND ADMINISTRATION OF DRUGS IN INTERCHANGEABILITY AND BIOCOMPARABILITY CLINICAL STUDIES.
- From 08:30 to 00:00 h, sampling as per the schedule of assessments.
- At 09:00 h, intravenous administration of 10 mL of 10% glucose solution.
- At 10:00 h Measurement of blood glucose and capillary glucose on a test strip (hemoglucotest), 2 hours post dose and intravenous administration of 10 mL of 10% glucose solution.
- Finger stick and IV blood glucose measurement 2 hours following dose.
- Finger stick and IV blood glucose, vital signs measurement 4 hours following dose.
- Finger stick for blood glucose determination and vital signs 8 hours following dose.
- As from 16:00 h, lunch.
- Finger stick and IV blood glucose, vital signs measurement 12 hours following dose.
- As from 21:00 h, dinner.

Second day:

- At 8:00 h, 24.0-h sampling.
- Finger stick for blood glucose determination, blood glucose and vital signs measurement 24 hours following dose.
- As from 9:00 h, breakfast.
- At 12:00 h, 28.0-h sampling.
- As from 15:00 h, lunch.
- Finger stick, IV blood glucose and vital signs measurement 32 hours following dose.
- At 16:00 h, 32.0-h sampling.
- As from 20:00 h, dinner.

Third day:

- At 8:00 h, 48.0-h sampling and safety laboratory tests.
- Physical examination.
- Vital signs measurement.
- Electrocardiogram.
- Approximately at 10:30 h, temporary discharge from the clinical unit.

Fourth day (ambulatory visit):

- At 8:00 h, 72.0-h sampling.

Fifth day (ambulatory visit):

- At 8:00 h, 96.0-h sampling.

Sixth day (ambulatory visit):

- At 8:00 h, 120.0-h sampling.

Seventh day (ambulatory visit):

- At 8:00 h, 144.0-h sampling.

Eight day (ambulatory visit):

- At 8:00 h, 168.0-h sampling.
- Approximately at 10:00 h, temporary discharge and free appointment.

8.2.2 Washout Period

Washout period lasts for 14 days after first dosing. It is intended to clear the prior dose before administering the next one and to fulfill with the NOM-177-SSA1-2013 criterion about having a period of at least 7 half-lives for the study drug.

8.2.3 Second Administration Period

Analogous to the First period of treatment after a 14 days wash-out period

8.2.4 End of Trial (EoT)

- At 08:00 h, 168.0-h sampling.
- Weight measurement for each subject.
- Blood for qualitative and quantitative pregnancy test in childbearing potential women.
- Blood sampling for safety laboratory tests.
- Urine sampling for safety laboratory tests.
- Physical examination.
- Vital signs measurement.
- Electrocardiogram.
- Approximately at 11:00 h, temporary discharge and free appointment.

8.3 Baseline demographic characteristics

At screening (within 21 days before first administration), the following demographic data will be collected: date of birth, sex (gender), race, and ethnicity
Specify any additional critical variables to be assessed such as:

- Information about previous and concomitant medications
- Medical history data including medication history

- Weight, height and BMI
- Special diets
- For women, menstrual status and date of last menstrual period.
- Month prior to drug administration, nicotine consumption
- Smoking history
- Alcohol consumption
- Caffeine or xanthine consumption -containing beverages, intake of grapefruit, orange, cranberry or juices of these three fruits, intake of food containing xanthine and usual diet.
- Physical examination, and serum virology
- Safety laboratory
- ECG
- Vital signs

8.4 Efficacy assessments

Not applicable.

8.5 Safety Assessments

Clinical safety will be assessed by means of a complete physical examination (at SCR, before each IMP administration and 48 and 168 h after each dose). On the other hand, throughout the study, subjects will be continuously monitored, including vital signs measurements (baseline and 4, 8, 12, 24, 32, 48 and 168 h post-dose) and electrocardiograms will be performed (baseline, 48 and 168 h post-dose). All examinations 168 h after second administration correspond to the EOT examination.

Safety will be assessed by means of full laboratory tests (at screening, at each baseline and 48 h after each administration) (hematology, clinical chemistry, coagulation, and urinalysis). In blood analyses besides glucose (baseline, 2, 4, 12, 24, and 32 post-dose), finger-stick glucose (Hematoglucotest) will be monitored (baseline, 2, 4, 8, 12 and 24 h post-dose), see flow chart.

Investigational drugs' safety profile will be assessed by means of the record, report and analysis of baseline medical conditions, including the use of drugs, smoking habits and alcohol use, adverse events, findings in the physical examination, including vital signs, drug abuse screening, pregnancy test and laboratory tests including hematology, clinical chemistry, coagulation, urinalysis).

Comprehensive assessment for any apparent toxicity experience by any subject should be carried out since informed consent signature and throughout the study. The Principal Investigator will report any adverse event, observed by him/her or the assigned medical staff or reported by the subject (see section 8.5.1.2). Reporting period for adverse events is described in section 8.5.1.3.

8.5.1 Adverse Event

8.5.1.1 Adverse Event Definitions

Adverse Event

An adverse event is any untoward medical occurrence which may occur during drug's clinical research stage but which do not necessarily has a causal relation with the drug. Therefore, an adverse event can be any untoward sign and symptom (including any abnormal finding in the laboratory tests), or condition temporarily related to the use of the drug (investigational), whether related or not to that product.

Adverse events associated to the use of drugs in humans, related or not to the drug, include the following:

- Any adverse event occurring during the use of the drug in the professional practice.
- An adverse event which occurs because of the abuse of drug products or substances.
- An adverse event which occur following the stop of drug's intake.
- An adverse event where there is a reasonable possibility that the event occurred only as a consequence of the subject's participation in the study (e.g., adverse event or serious adverse event due to the stop of anti-hypertensive drugs during wash-out phase) should be reported as an adverse event even though this is not related to the investigational product.
- Clinical onset on any failure of the expected pharmacological action.

In case the event meets the criteria for "serious" adverse event, it should be recorded and reported as that.

8.5.1.2 Adverse Event's Causality with the Investigational Product

Adverse events' causality with study drug is a clinical decision based on all available information at the moment of the adverse event onset.

Adverse reactions are classified according to the causality according to the following probabilistic categories (NOM-220-SSA1-2012):

A causality assessment would include.-

- **Certain.** It consists of an event (clinical onset or an abnormal result of laboratory test) which occurs within a reasonable period of time following the drug's administration and which cannot be explained by the natural condition's evolution, by any concomitant pathology or by other drugs dosing. Response to drug's stop should be clearly evident.

- **Probable.** It consists of an event (clinical onset or an abnormal result of laboratory test) which follows a reasonable period of time following the drug's administration and which can hardly be related to the natural condition's evolution, concomitant pathologies or to other drugs dosing. A clinically reasonable response is obtained upon stop of other(s) drug(s).

- **Possible.** It consists of an event (clinical onset or an abnormal result of laboratory test) which follows a reasonable period of time following the drug's administration and which can hardly be related to the natural condition's evolution, concomitant pathologies or to other drugs dosing. There is no information available related to the suspect drug's dosing or this is not clear.

- **Suspect.** It consists of a an event (clinical onset or abnormal laboratory test) which follows a reasonable sequence of time following the drug's dosing which makes causality unlikely (but not impossible). It might be explained in a reasonable way since it is part of the natural evolution of the condition, or it is due to the presence of concomitant pathologies or other drugs dosing.

- **Conditional - Unclassifiable** it consists of an event (clinical onset or abnormal laboratory test result) which cannot be properly assessed since more data are required or because additional data are still being analyzed.

- **Not evaluable - Unclassifiable** It consists of a report which suggest that there is an adverse reaction which cannot be assessed since collected information is not enough or inconsistent. The report cannot be completed or checked.

Investigators must also systematically assess the causal relationship of AEs to metformin/Gliclazide using the following definitions. Decisive factors for the assessment of causal relationship of an AE to the metformin/Gliclazide include, but may not be limited to, temporal relationship between the AE and the metformin/Gliclazide treatment, known side effects of metformin/Gliclazide, medical history, concomitant medication, course of the underlying disease, trial procedures.

- **Unrelated:** Not reasonably related to the metformin/Gliclazide treatment. AE could not medically (pharmacologically/clinically) be attributed to the IMP/study treatment under study in this clinical trial protocol. A reasonable alternative explanation must be available.
- **Related:** Reasonably related to the IMP/study treatment. AE could medically (pharmacologically/clinically) be attributed to the IMP/study treatment under study in this clinical trial protocol.

Other factors to be considered for adverse event's causality assessment with the study drug are:

- Recovery or discontinuation, relapse or rechallenge: Subject's response following stopping the drug or subject's response following rechallenge should be considered based on the usual clinical course of the corresponding event.
- Response pattern known for this type of drug: Clinical/Pre-clinical
- Exposure to physical and/or mental stress: Exposure to stress may produce adverse changes in the receptor and provide a rationale and a better explanation for the event.

Test drug's pharmacology and pharmacokinetics: Test drug's pharmacokinetic properties (absorption, distribution, metabolism and excretion) as well as the subject's individual pharmacodynamics should be considered.

8.5.1.3 Adverse Event Severity

Adverse events intensity or severity should be classified as follows (NOM-220-SSA1-2012):

- Mild
  They occur with easily tolerated signs and symptoms. They do not require treatment or extend hospitalization, and do not necessarily require drug's discontinuation.
- Moderate
  They interfere with usual activities (may cause work or school absenteeism) without affecting patient's life directly. They require pharmacological therapy and do not necessarily require the discontinuation of the drug which caused the event, reaction, or suspect adverse reaction.
- Severe
  They interfere with usual activities (may cause work or school absenteeism). They require pharmacological therapy and the discontinuation of the drug which caused the event, reaction, or suspect reaction.

8.5.1.4 Laboratory Abnormal Findings

Abnormal laboratory findings and other abnormal findings during the study (e.g., ECG trace) should not reported as serious adverse events, unless they are related to clinical signs and symptoms, which cause treatment's discontinuation or, in the other hand, they are considered clinically important by the Principal Investigator. In the event laboratory abnormality meets these criteria, the identified medical condition (for instance, anemia, and high ALT) should be reported as an adverse event instead of the abnormal value by itself.

8.5.1.5 Unexpected Adverse Event

An unexpected adverse event is any adverse event which specificity or severity is not mentioned in the most recent product's information (Investigator's Brochure - or package insert for marketed products). Likewise, reports which provide important information about specificity or severity of a known adverse event, already documented, constitute unexpected adverse events. For instance, any event which is more specific or more severe than those described in the Investigator's Brochure should be considered "unexpected".

8.5.1.6 Adverse Events of Special Interest

Hypoglycemia is a condition characterized by abnormally low blood glucose levels, usually less than 70 mg/dl and may be associated with the following symptoms: headache, increase appetite, nausea, vomit, tiredness, drowsiness, sleep disorders, restlessness, aggressiveness, reduced concentration, wakefulness and reaction; depression, confusion; visual and speech disorders; aphasia; tremors, paresis, sensory disorders, dizziness, helplessness feeling, lack of self-control, delirium, seizures, shallow breathing, bradycardia, drowsiness, syncope which might lead to coma and death.

Subjects will be monitored closely in the event of a nonserious AESI, the investigator will complete the AESI Report Form and send it to the Sponsor. Names, addresses, and telephone and fax numbers for AESI reporting will be included on the Report Form. Serious AESIs have to be reported in an expedited manner as SAEs as outlined above.

8.5.1.7 Serious Adverse Event

A serious adverse event is any unexpected medical occurrence which at any dose:

- Results in death.
- It is life-threatening.
- Requires hospitalization or prolongs the existing hospitalization.
- Results in persistent or important disability or incapacity.
- It is a congenital abnormality or birth defect.
- It is clinically important event.

Some clinically important events, although they do not lead to death, are not life-threatening or require hospitalization, may be considered serious adverse events based on the appropriate medical judgment and the need of medical or surgical care to avoid the events previously mentioned in this definition. Examples of those medical events include: allergic bronchospasm which require intensive care in the emergency room or at home, blood dyscrasias or seizures which require hospitalization, or the development of drug's dependence or abuse.

Life-threatening means that the subject was in danger, as per Investigator's judgment, immediate risk of death due to the reaction when it occurred.

Disability means an important disorder in the capacity of a person to perform usual functions or activities.

For reporting purposes, any suspect of transmission of an infective agent by any of the study drugs is considered a serious adverse event, as described in section 8.5.1.4.

8.5.1.8 Recording Methods and Adverse Events Assessment

Any adverse event occurring during the study period should be recorded in the medical chart as well as in the case report form for each subject. This information will be obtained from the subject of the study, which will be asked directly about the adverse event.

Documentation should be supported by a record in the subject's medical chart. Any abnormality in laboratory tests, considered clinically relevant, for instance, those which lead to the subject's withdrawal from the study, require treatment, causing evident clinical onset in the subject, considered relevant by the Investigator, should be reported as an adverse event.

All adverse events should be described in detail, including subject's identity (name, age and gender), adverse event, suspected drug, reporter's data, onset and end date for the event, event's name and treatment, generic and brand name, dosage, route of administration, reason of prescription, event's consequences and relevant data in the medical history, this information must be consistent with the CRF, the SAE and AESI Report Forms (as applicable).

8.5.1.9 Definition of Reporting Period for Adverse Events

Report of adverse events will start as per the informed consent form signature and until EOT.

8.5.1.10 Procedure for Reporting Adverse Events and Serious Adverse Events

Report of adverse events will be the responsibility of the PPD . and it will be made according to the standard operating procedure, code: PPD , REPORT OF CLINICAL AND BIOAVAILABILITY STUDIES ADVERSE EVENTS

Report of adverse events will be made according to the standard operating procedure, code: PP , REPORT OF CLINICAL AND BIOAVAILABILITY STUDIES ADVERSE EVENTS and the Mexican Official Standard for the Setting-up and Operations of Pharmacovigilance in Mexico, NOM-220-SA1-2012. Therefore, any serious adverse event or suspect serious adverse reaction should be notified to the National Center of Pharmacovigilance (CNFV) from the Federal Commission for the Protection against Sanitary Risks (COFEPRIS) as well as to the Sponsor within the first 24 h following the Investigator is aware of it. A supplemental report, as detailed as possible which include all information collected, should be sent within 15 days. Report to the

CNFV will be made by the Pharmacovigilance Officer at the PPD To comply with NOM-220-SSA1-2012, mild or moderate, expected and unexpected adverse reactions should be reported by the study Principal Investigator at the PPD along with the clinical stage report to the CNFV.

Means to notify the Sponsor are the following:

Fax: PPD

Telephone:PPD

Mobile: PPD

E-mail:PPD

In the event of any new SAE occurring during the reporting period, the Investigator must immediately (within a maximum of 24 HOURS after becoming aware of the event) inform the Sponsor or its designee in writing. All written reports should be transmitted using the SAE Report Form, which must be completed by the Investigator following specific completion instructions.

In exceptional circumstances, an SAE (or follow-up information) may be reported by telephone; In these cases, a written report must be sent immediately thereafter by fax or e-mail. Names, addresses, and telephone and fax numbers for SAE reporting will be included in the trial-specific

SAE Report Form.

Relevant pages from the CRF may be provided in parallel (for example, medical history, concomitant drugs). Additional documents may be provided by the Investigator, if available (for example, laboratory results, hospital report, and autopsy report). In all cases, the information provided on the SAE Report Form must be consistent with the data about the event recorded in the CRF.

The Investigator must respond to any request for follow-up information (for example, additional information, outcome, final evaluation, other records where needed) or to any question the Sponsor/designee may have on the AE within the same timelines as those noted above for initial reports. This is necessary to ensure prompt assessment of the event by the Sponsor or designee and (as applicable) to allow the Sponsor to meet strict regulatory timelines associated with expedited safety reporting obligations.

Requests for follow-up will usually be made via the responsible Monitor, although in exceptional circumstances the Global Drug Safety department may contact the Investigator directly to obtain further information or to discuss the event.

The reports to Merck shall be sent in English using the Merck SAE form via fax or email within 24 hours.

Fax No.: PPD, email address: PPD.

The Investigator should answer any request of follow-up information (for instance, additional information, outcome, final assessment, and other records, whenever it is necessary) for any other question the Sponsor or designated person might have regarding the adverse event, within the same time periods aforementioned. This is necessary to ensure an expedited assessment of the event by the Sponsor or designated person and (as appropriate) to allow that the Sponsor meets stringent official deadlines with regard to the duty of reporting safety information in an expedited way.

8.5.1.11 Report of Safety Information to the Health Authorities, Ethics and Investigation Committee and Investigational Committee

According to local law and regulation, all serious and non-serious adverse events should be reported to the appropriate Ethics and Investigation Committee, Investigational Committee and to the Health Authorities. This will be carried out according to the standard operating procedure, code: PPD , REPORT OF CLINICAL AND BIOAVAILABILITY STUDIES ADVERSE EVENTS

The Investigator must comply with any applicable site-specific requirements related to the reporting of SAEs (particularly deaths) involving trial subjects to the IEC/IRB that approved the trial.

In accordance with ICH GCP, the Sponsor/designee will inform the Investigator of "findings that could adversely affect the safety of subjects, impact the conduct of the trial or alter the

IEC's/IRB's approval/favorable opinion to continue the trial." In particular and in line with respective regulations, the Sponsor/designee will inform the Investigator of AEs that are both serious and unexpected and are considered to be related to the administered product ("suspected unexpected serious adverse reactions" or SUSARs). The Investigator should place copies of Safety Reports in the Investigator Site File. National regulations with regard to Safety Report notifications to Investigators will be taken into account.

When specifically required by regulations and guidelines, the Sponsor/designee will provide appropriate Safety Reports directly to the concerned lead IEC/IRB and will maintain records of these notifications. When direct reporting is not clearly defined by national or site-specific regulations, the Investigator will be responsible for promptly notifying the concerned IEC/IRB of any Safety Reports provided by the Sponsor/designee and of filing copies of all related correspondence in the Investigator Site File.

For trials covered by the European Directive 2001/20/EC, the Sponsor's responsibilities regarding the reporting of SAEs/SUSARs/Safety Issues will be carried out in accordance with that Directive and with the related Detailed Guidance documents.

8.5.1.12 Monitoring of Subjects with Adverse Events

Adverse events are recorded and assessed on an ongoing basis throughout the study, and they will be assessed for the final outcome in the 14-day follow-up following the last dose of the drug.

All ongoing adverse events, including the follow-up period, should be watched and followed-up by the Investigator until they are stable or until their outcome is known; unless it is documented that the subject is "lost to follow-up". All reasonable efforts should be made to collect information and these should be documented. It is also the Principal Investigator's responsibility to ensure that all additional and necessary therapeutic measures are taken as well as the follow-up procedures.

8.5.2 Pregnancy and exposure to drugs in the Uterus

All pregnancies with a conception date estimated during the period defined in section 9.5.1.3. will be recorded in the appropriate CRF page/section for female subjects and male subjects' female partners as AE or SAE depending on the outcome of the pregnancy The Principal Investigator should inform immediately the Sponsor or designated person about any pregnancy using a Pregnancy Report Form which should be submitted according to the same procedure for SAE referred in section 9.5.1.4.

The Principal Investigator or designated person should follow-up, document and report all pregnancies outcomes, even if subjects were withdrawn from the study.

The Principal Investigator should inform the Sponsor or designated person about the outcome using the Pregnancy Report Form. In case of an abnormal outcome, the SAE Report Form will be used. In case subject child/fetus experienced an event, the Parent-Child/Fetus Adverse Event Report Form will be used.

Any abnormal outcome should be reported immediately as described in section 9.5.1.4. All outcomes should be reported 45 following the delivery.

In case of pregnancy during the study, the subject should be withdrawn and study drug should be immediately withdrawn. Sponsor or designated person should be immediately informed and follow-up should be made as aforementioned.

Uncontrolled diabetes during pregnancy (gestational or permanent) is associated with increased congenital abnormalities and perinatal mortality. The use of the combination of metformin and Gliclazide has not been studied in pregnant women.

A limited amount of data from the use of metformin in pregnant women does not indicate an increased risk of congenital abnormalities. Animal studies with metformin do not indicate harmful effects with respect to pregnancy, embryonic or fetal development, parturition or postnatal development. With Gliclazide no teratogenic effects have been shown in animal studies, but lower fetal body weight was observed in animals receiving doses 25 fold higher than the maximum recommended dose in humans.

No impairment of fertility was seen in a study with male and female rats.

When the patient plans to become pregnant and during pregnancy, it is recommended that diabetes is not treated with, but insulin should be used to maintain blood glucose levels as close to normal as possible.

8.5.3 Clinical Laboratory Assessments

Blood and urine samples for safety lab (biochemistry, glucose, hematology; coagulation times, urinalysis), and for pregnancy tests will be collected at Screening and EOT as well as described in the following and according to the schedule of assessments (Appendix 1).

- **Blood samples for hematology:**

Two (2) blood samples will be collected per period (pre-dose and 48.0 h post-dose) in test tubes with $EDTA_{K2}$, specifically designed for venipuncture (hemoglobin, hematocrit, erythrocytes, mean cell volume, mean corpuscular hemoglobin, white blood cells, platelets, neutrophils, lymphocytes, monocytes, eosinophils and basophils). Dosing and blood sampling sequence among subjects should be kept the same for all subjects according to the times for these activities. Exact time when samples have been taken should be recorded in the appropriate forms. Once the sample from the last group is taken, it will be taken to the sample processing area and kept at room temperature until their shipment to the analytical unit. Blood from these subjects will be collected as described in the following:

- Two (2) blood samples will be collected for hematology per period at times established in the schedule. Test tubes with $EDTA_{K2}$, specifically designed for venipuncture, will be used.
- Each sample will be 6 mL. Blood withdrawn to clean catheter should be disposed prior to the blood sample collection (purge).
- A ± 1 minute window is allowed for sampling.
- Samples should be maintained at room temperature (between 20 and 25 °C [68 °F to 77 °C).
- Blood tubes will be perfectly labeled with Study No. (##), Case No. (C##), Sample No. (S##) and Visit No. (V#) according to the SOP, code, PPD , TUBES LABELING AND DISTRIBUTION IN RACKS; PPD COLLECTION OF MULTIPLE BLOOD SAMPLES FOR INTERCHANGEABILITY AND BIOCOMPARABILITY STUDIES. They will be kept at room temperature until their delivery to the clinical laboratory.

- **Blood samples for blood chemistry and glucose determination:**

Two samples will be collected per period for blood chemistry (albumin, alanine aminotransferase, aspartate aminotransferase, protein total, total bilirubin, conjugated bilirubin, unconjugated bilirubin, globulin, albumin/ globulin ratio, blood urea nitrogen, glucose, urea, creatinine, uric acid, cholesterol and triglycerides) (pre-dose and 48 h post-dose) and 5 samples for glucose determination (2, 4, 12, 24 and 32 h post-dose) in test tubes specifically designed for venipuncture without anticoagulant. Finger stick glucose measurement (Hematoglucotest) will be made pre-dose and 2, 4, 8, 12 and 24 h post-dose per period. Dosing and blood sampling sequence among subjects should be kept in such a way that times are the same for these activities for all subjects. Exact time when samples have been taken should be recorded in the appropriate forms. Once the sample from the last group is taken, it will be taken to the sample processing area and kept at - 20

°C (- 4 °F) until their shipment to the analytical unit. Blood from these subjects will be collected as described in the following:

- Each sample will contain 6 mL for blood chemistry and 3 mL for glucose determination. Blood withdrawn to clean catheter should be disposed prior to the blood sample collection (purge).
- A ± 1 minute window is allowed for sampling.
- Each sample will be centrifuged at 2500x g for 15 minutes at 4 °C (39.2 °F) (± 2 °C [35.6 °F]), to obtain at least 1 mL of serum for blood chemistry and 1 mL of serum only for glucose determination.
- Blood tubes will be labeled with Study No. (##), Case No. (C##), Sample No. (S##) and Visit No. (V#) according to the SOP, code, PPD , TUBES LABELING AND DISTRIBUTION IN RACKS; PPD COLLECTION OF MULTIPLE BLOOD SAMPLES FOR INTERCHANGEABILITY AND BIOCOMPARABILITY STUDIES. They will be kept at - 20 °C (- 4 °F) until their delivery to the clinical laboratory.

- **Blood samples for coagulation:**

Two (2) blood samples per period will be collected for coagulation times (pre-dose and 48 h post-dose) in test tubes with sodium citrate specifically designed for venipuncture (prothrombin time and partial thromboplastin time). Dosing and blood sampling sequence among subjects should be kept in such a way that times are the same for these activities for all subjects. Exact time when samples have been taken should be recorded in the appropriate forms.

Once the sample from the last group is taken, it will be taken to the sample processing area and kept at - 20 °C (- 4 °F) until their shipment to the analytical unit. Blood from these subjects will be collected as described in the following:

- Each sample should have 3 mL to determine coagulation times. Blood withdrawn to clean catheter should be disposed prior to the blood sample collection (purge).
- A ± 1 minute window is allowed for sampling.
- Each sample will be centrifuged at 2500 x g for 15 minutes at 4 °C [39.2 °F] (± 2 °C [35.6 °F]) to obtain at least 1 mL of plasma.
- Blood tubes will be labeled with Study No. (##), Case No. (C##), Sample No. (S##) and Visit No. (V#) according to the SOP, code, PPD , TUBES LABELING AND DISTRIBUTION IN RACKS; PPD COLLECTION OF MULTIPLE BLOOD SAMPLES FOR INTERCHANGEABILITY AND BIOCOMPARABILITY STUDIES. They will be kept at - 20 °C (- 4 °F) until their delivery to the clinical laboratory.

- **Urine samples for urinalysis / microscopic analysis / sediment:**

Two (2) urine samples per period will be collected (pre-dose and 48 h post-dose) in containers specifically designed. Time when samples have been taken should be recorded in the appropriate forms. Once the sample from the last subject is taken, it will be taken to the sample processing area and kept in the fridge until their shipment to the clinical laboratory.

Urine samples will be taken from fresh, spontaneous urine from each subject. The following parameters will be evaluated by Aution max 4280 Urine analyzer:

- pH, specific gravity
- Proteins*, glucose, ketones, nitrites*, bilirubine, urobilinogen, blood*, leucocytes*
- * if positive, a microscopic examination (urine sediment) will be performed.
- Urine sample from these subjects should be kept in the fridge.

Containers will be labeled with Study No. (##), Case No. (C##), Sample No. (S##) and Visit No. (V#) according to the SOP, code, PPD , SAMPLING FOR CLINICAL LABORATORY FOR SUBJECTS' SCREENING IN INTERCHANGEABILITY AND BIOCOMPARABILITY STUDIES . They will be kept in the fridge until their delivery to the clinical laboratory.

The Sponsor should receive a list of laboratory normal ranges before shipping the study drug. Any change to the study laboratory normal ranges should be submitted to the Sponsor.

- **Pregnancy test:**

For all women of childbearing potential one blood sample per period will be collected for pregnancy tests (qualitative and quantitative) at day -1 of each treatment period in test tubes specifically designed for venipuncture without anticoagulant.

5 mL of blood will be collected and processed as follows:

- Each sample will be centrifuged at 2500 x g for 15 minutes at 4 °C [39.2 °F] (± 2 °C [35.6 °F]) to obtain at least 1 mL of plasma.
- Samples should be maintained at room temperature (between 20 and 25 °C [68 °F to 77 °C).
- Blood tubes will be labeled with Study No. (##), Sample No. (S##) and Visit No. (V#) according to the SOP, code, PPD , TUBES LABELING AND DISTRIBUTION IN RACKS; PPD COLLECTION OF MULTIPLE BLOOD SAMPLES FOR INTERCHANGEABILITY AND BIOCOMPARABILITY STUDIES. They will be kept at room temperature until their delivery to the clinical laboratory.
- Pregnancy qualitative test will be performed in the clinical unit with the One Step HCG insta test trip (serum), for In-Vitro diagnostic.
- Pregnancy quantitative test will be performed in the clinical laboratory with the architect i 2000 analyzer by Chemiluminescence.

8.5.4 Vital signs, ECGs, Physical Examinations and other Assessments

At Screening, EOT and prior to each dosing, blood pressure, pulse, respiratory rate and forehead temperature (subjects should be in rest and in supine position at least 10 minutes before vital signs measurement; and they should be in rest and in supine position during the vital signs measurements) will be measured.

These values should be within the normal range (SBP between 80 and 129 mmHg and DBP between 50 and 89 mmHg) in order the subject is allowed to keep on participating in the study. Same measurements will be made during all shifts following dosing at each one of the visits. Vital signs will be measured during the 2 cross over periods as shown in the following:

- **Record of Vital Signs**

First Visit	Second Visit
Admission	Admission
Pre-dose	Pre-dose
Morning (4 hours post-dose approximately)	Morning (4 hours post-dose approximately)
Evening (8 hours post-dose approximately)	Evening (8 hours post-dose approximately)
Night (12 hours post-dose approximately)	Night (12 hours post-dose approximately)
24 and 32 hours post-dose approximately	24 and 32 hours post-dose approximately
Discharge (48 hours post-dose approximately)	Discharge (48 hours post-dose approximately)
Ambulatory samples (72, 96, 120, 144 and 168 hours post-dose approximately)	Ambulatory samples (72, 96, 120, 144 and 168 hours (EOT) post-dose approximately)

In the event any subject has clinically significant changes out of normal ranges for blood pressure and pulse during confinement post-dose, he/she will be closely observed by the medical staff. Supportive measures, as appropriate, will be taken according to the current subject's condition until they are resolved. If change persists, continuation in the study will be considered by the Principal Investigator.

At Screening, EOT and prior to each dosing and 48 hours after each IMP administration, electrocardiograms will be measured.

8.6 Pharmacokinetics

Twenty two (22) 10-mL samples of venous blood will be taken per period with catheter or venipuncture for the measurement of drug's plasma concentration: -1.5 (Pre-treatment, control), 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0, 12.0, 16.0, 24.0, 28.0, 32.0, 48.0, 72.0, 96.0, 120.0, 144.0 and 168.0 h following treatment.

- **Pharmacokinetics Blood Samples**

- Two (2) blood samples will be collected per period at times established in the schedule. Test tubes with sodium heparin, specifically designed for venipuncture, will be used.

- Each sample will be 10 mL. Blood withdrawn to clean catheter should be disposed prior to the blood sample collection (purge). Blood volume to be withdrawn by visit will be of 220 mL approximately (440 mL a month), 880 mL of blood in total at the end of the study.

- A ± 1 minute window is allowed for sampling during absorption and distribution phase, and up to ± 3 minutes during the elimination phase.

- Each sample will be centrifuged at 2500 x g for 5 minutes at 4 °C [39.2 °F] (± 2 °C [35.6 °F – 42.8°F]).

- Resulting plasma volume will be transferred into four 1.5 or 2 mL cryotubes. For each analyte one tube serves as back-up sample.

- Plasma tubes will be identified with Study No. (##), Case No. (C##), Sample No. (S##) and Visit No. (V#). They will be kept frozen at a temperature lower than or equal to - 40 °C or lower, according to the standard operating procedures, code: PPD , EARLY ORGANIZATION FOR THE CONDUCT OF AN INTERCHANGEABILITY AND BIOCOMPARABILITY STUDY; and PPD : PROCESSING AND PACKAGING OF BIOLOGICAL SAMPLES FOR INTERCHANGEABILITY AND BIOCOMPARABILITY STUDIES, PPD : SAMPLES TRANSPORTATION FROM THE CLINICAL UNIT TO THE ANALYTICAL UNIT; until their delivery at the analytical unit.

▪ Blood Sampling Schedule

Clinical Phase

NUMBER OF SUBJECTS:	22	GENDER:	Female and male
BIOLOGICAL MATRIX:	Plasma	DOSAGE FORM:	Tablet
FORMULATION:	MR Tablet of fixed dose combination of Metformin 1000 mg and Gliclazide 30 mg	DOSE AND DOSAGE UNITS:	Metformin 1000 mg/Gliclazide 30 mg (one MR tablet)
WASH-OUT TIME:	14 days	ANTI-COAGULANT:	Sodium heparin
BLOOD VOLUME WITHDRAWN BY SAMPLE:	10 mL	TOTAL OF SAMPLES BY SUBJECT IN EACH PERIOD:	22

Sampling Interval

Phase	Sample	Time	Unit
--	0	-1.500	h
A	1	0.5	h
A	2	1.00	h
A	3	2.00	h
~ C_{max}	4	3.00	h
~ C_{max}	5	4.00	h
~ C_{max}	6	5.00	h
~ C_{max}	7	6.00	h
E	8	7.00	h
E	9	8.00	h
E	10	10.00	h
E	11	12.00	h
E	12	16.00	h
E	13	24.00	h
E	14	28.00	h
E	15	32.00	h
E	16	48.00	h
E	17	72.00	h
E	18	96.00	h
E	19	120.00	h
E	20	144.00	h
E	21	168.00	h

Total Number of Samples			
No. of Cases	Samples	Periods	Total
22	22	2	968

Diet
Standardized by the clinic

Each subject will participate for around 180 h (48 h confined and with outpatient follow-up for samples from 72.00, 96.00, 120.00, 144.00 and 168.00 h) for each of the periods and an approx. 154 h period within the 14-day wash-out period. The wash-out period starts per the time of drug's intake in the first period and thus includes the 168 h period for sampling and the approx. 14 h period on day -1 to day 1 of the second period (until second administration).

- **Samples Shipment**

To ensure transportation conditions of biological samples from the laboratory, where they were processed for freezing and storage, to the analytical unit do not affect samples' stability, it is essential to comply with the following guidelines:

- The Study Principal Investigator or the Sub-Investigator will define shipment's date and time in accordance with the analytical unit, as well as the transportation to be used and approximate time for itinerary.
- Mechanism of control and security for samples shipment will be defined by the person in charge of transportation in agreement with the Principal Investigator or Sub-Investigator.
- The Samples Processing and Pharmacy Coordinator or a designated person will sign a list of material to be shipped, with a detailed description by subject for tubes quantity and codes.
- An internal temperature reading system will be available in the freezer. A reading will be made at least every 15 minutes with its appropriate record.
- In addition, reading at the moment of departure of the material from the samples processing area, as well as a reading at the reception at the analytical unit will be made.
- Samples transportation is the responsibility of the clinical unit where samples collection and processing took place.
- Delivery will be made personally, by a member of the Clinical Unit.
- All samples should arrive deep frozen (- 20 °C [- 4.0 °F] as maximum).

- **Samples Receipt, Check and Storage by the Analytical Unit**

- The Person responsible for the study or designated person from the analytical area will receive samples (along with documentation) and check 100 % with the assistance from analysts and Quality Assurance. They will record any remarks on the appropriate form. Once review is finished the person responsible for the study or designated person will store samples in the ultra-freezer documenting it on the equipment's notebook.
- Quality Assurance will confirm accuracy and reliability of operations.

Samples which do not meet acceptance criteria will be rejected, according with the Manual of Operations Reception, storage, handling matrix and biological samples.

(see Manual of Operations). In addition to those which don't have documentation along with them. In such instances, the clinical unit should be informed about the reason why samples were rejected. Rejected biological specimens will be kept at the analytical unit until the analytical unit and the clinical unit make an agreement about their disposition.

Further details e.g. on the collection, processing, labeling, and shipping of the samples will be detailed in the trial Manual of Operations (MOP).

- **Handling of Biological Specimens by the Analytical Unit**

Biological specimens will be handled and processed as described in the appropriate analytical technique.

- **Biological Specimens Disposition**

As agreed with the Sponsor or 30 calendar days following the final report, samples will be removed from the ultra-freezer and will be stored in a transient warehouse for infectious biological hazard residues until they are collected for their final disposition by an authorized designated company.

- **Ultra-frozen Samples Labeling**

Cryotubes will be identified with labels according to the standard operating procedure, code: PP [redacted], EARLY ORGANIZATION FOR THE CONDUCT OF AN INTERCHANGEABILITY AND BIOCOMPARABILITY STUDY. Each cryotube will state the study number, subject's number, period and number of samples specified in the protocol. Labels will be digitally designed and printed using thermal printer. Label and printing type will ensure that that printing and adhesion resist several freezing and thaw cycles.

9 Pharmacokinetic and statistical analysis

Pharmacokinetics and statistical analysis of Metformin and Gliclazide's plasma concentrations will be conducted by the analytical unit from the PPD [redacted], according to their standard operating procedures and in accordance with the NOM-177-SSA1-2013.

Pharmacokinetic parameters (non-compartmental analysis) and statistical analysis for determining the food effect on bioavailability will be calculated using Phoenix WinNonlin 6.3 software.

9.1 Sample Size

According to the goals for the study treatment regarding postprandial/fasting, pharmacokinetic parameters will be calculated AUC and C_{max} for both analytes, Metformin and Gliclazide. In order to have an accurate and reasonable precision for the estimated ratios, 22 subjects will be randomized.

Taking 90 % confidence interval for the postprandial/fasting ratio as a measure for accuracy and assuming an average variation coefficient of 20 % for AUC (AUC0-∞, AUC0-t) and C_{max} for Metformin or Gliclazide, then with 18 evaluable subjects the food effect on pharmacokinetics can be estimated with an accuracy of 12 %. The following ratio will be carried out, being R the estimated ratio and LCL / UCL lower and higher confidence levels for the 90% confidence interval for R:

$$0.89 * R \leq LCL < UCL \leq 1{,}12 * R$$

An accuracy of 12% will result in an accurate description for the food effects. Besides, 18 evaluable subjects are enough to detect a difference among treatments of 18 % or higher with a power of 80 % at least. Taking into account a drop-out rate around 20 %, 22 subjects should be randomized.

9.2 Randomization

Valid statistical inferences are generally derived by assuming that errors in the observations are distributed independently, in random variables. Randomization generally ensures validity of this hypothesis. Full randomization of subjects to the treatment's sequence will be made according to the design sequences based on the internal procedures from the PPD compliant with the Mexican Official Standard NOM-177-SSA1-2013 using the randomization.com software.

A total of 22 subjects are to be enrolled in the trial and randomized to one of 2 treatment sequences, such that 11 subjects are assigned to each treatment sequence. Male and female subjects will be included. Every effort will be made to reach gender balance (at least 40 % for each gender).

Once it is confirmed that all inclusion criteria and none exclusion criteria have been met, a consecutive number in ascendant order will be assigned to subjects, starting by 1 up to the last case (22), as described in sections 7.3 and 7.4, and according with the SOP code: PPD, RANDOMIZATION IN AN INTERCHANGEABILITY AND BIOCOMPARABILITY CLINICAL STUDY.

9.3 Outcome variables

9.3.1 Main outcome variable

To assess the food effect on bioavailability the pharmacokinetics metrics going to be $AUC_{0-\infty}$, AUC_{0-t} and C_{max} for Metformin and Gliclazide, given as single dose in fasting state and postprandial conditions.

9.3.2 Secondary outcome variables

- Additional pharmacokinetic parameters will be determined: $t_{1/2}$, t_{max}, Vz/f and CL/f.
- To assess and compare safety and tolerability of the fixed dose combination metformin /Gliclazide administered in fasting and fed state.

9.4 Deviation and Exclusion to the Statistical Analysis

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 not be replaced. 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 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.

9.5 Statistical Analyses Description

Descriptive statistical methods will be used to summarize demographic characteristics, pharmacokinetics parameters and adverse events.

Individual plasma concentration-time will be tabulated and plotted. Primary pharmacokinetics parameters, AUC and C_{max}, 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.

Statistical Analysis

This trial intends to estimate the effect of food on pharmacokinetics. It is not intended to prove or confirm statistically the lack of any food effect.

Therefore, no statistical hypothesis is presented. As primary analysis, a mixed model will be applied for all C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for Metformin and C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for Gliclazide, of the fixed dose combination metformin /Gliclazide log transformed. With TREATMENT, PERIOD and SEQUENCE as fixed effects, and the SUBJECT (SEQUENCE) as random effect.

Based on the residual error term 90 % confidence intervals will be built for the estimated differences postprandial - fasting, resulting in 90 % confidence intervals for postprandial/fasting ratios following the repeated transformation.

Statistical comparisons between treatments A and B, with respect to C_{max}, AUC_{0-t}, and AUC_{∞} of Metformin/Gliclazide, will in each case separate based on a mixed linear model for log transformed (natural log) pharmacokinetic parameter data. For each parameter evaluation and each pairwise comparison, the statistical model will include covariate adjustments for period and sequence as fixed effects, and subject, nested within sequence as random effect. Carry-over effects will be assumed ignorable.

General linear model for the analysis of variance, representing the experimental design, will be used for the analysis of data for pharmacokinetic variables by considering the additive form for the following variation sources:

- Dosing sequence.
- Subjects nested in the dosing sequence called variability or residual value between subjects.
- Dosing period.
- Treatment or formulation.
- Experimental error, called variability or residual value between subjects.

That general linear model, applied to the crossed designs to determine the food effect on bioavailability is as follows:

$$Y_{ijk} = \mu + G_k + S_{jk} + P_j + F_{(j,k)} + e_{ijk}$$ (Chow & Liu, 2009)

Where:

μ = overall mean

G_k = fixed effect of k^{th} sequence

S_{jk} = randomized effect of the i^{th} subject in the k^{th} sequence, where i = 1,2,..., n_k y k = 1,2,...K

P_j = fixed effect of the j^{th} period, where j = 1,2,..., J y $\Sigma_j P_j = 0$

$F_{(j,k)}$ = fixed direct effect of the formulation in the k^{th} sequence, which is administered in the j^{th} period and $\Sigma F_{(j,k)} = 0$

e_{ijk} = is the randomized error (intra-subject) in the Y_{ijk} observation

The sequence effect should be assessed using the subject's square means nested in the sequence as error term. All other major effects should be assessed against the residual error (error square means) and report the F values respectively. Besides, it should be stated if the variation source is significant, whenever p is less than 0.05 ($p < 0.05$).
Should significant effect is observed in the variability factors for sequence or periods, NOM-177-SSA1-2013 will be followed.

9.6 Extreme Values

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 the model used in the design.

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

10 Ethical and Regulatory Issues

10.1 Principal Investigator's Responsibilities

The Principal Investigator is responsible for the study conduct at the site. He/she will make sure that the study is conducted according to the clinical trial protocol, ethical principles established in the Declaration of Helsinki, ICH, GCP and established regulation NOM-177-SSA1-2013, which states tests and procedures to show that a drug is interchangeable as well as the requirements which Authorized Third-Parties should fulfill to perform the tests. The Investigator should ensure that only subjects who have provided their informed consent are included in the study.

10.2 Information for the Subject and Informed Consent

An unconditional requirement for each subject before their participation in the study is to have a written informed consent, which should be given before any study-related activity is carried out.

Therefore, the Principal Investigator or designated personnel should provide appropriate information before obtaining informed consent.

Subject's Information Sheet should be elaborated in Spanish, according to the ICH GCP and will be provided by the Sponsor with the purpose to get the informed consent. Besides providing written information to the potential subject, Investigator or designated person will inform them verbally all relevant aspects of the study, using language chosen so that information can be fully and easily understood by the subjects. Subject will be given enough time to read the information and ask questions and request additional information and explanations.

After providing information to the subject, informed consent form should be signed and dated by the subject, the Principal Investigator and two witnesses.

The dated and signed informed consent will be kept in the study site and should be securely filed so that files can be retrieved at any moment for monitoring, auditing and inspection purposes. A copy of the signed and dated informed consent form should be provided to the subject before their participation in the study.

Whenever new relevant information arises for the informed consent, the Investigator will revise subject's information and any other written information to be provided to the subjects, which should be submitted to the Committees for review and approval.

Revised and approved version of the written information will be used. The Principal Investigator or designated personnel will explain each subject all changes made to the previous version and will obtain a new written consent to continue his/her participation in the study. Subject will be given enough time to read the information and ask questions, and request additional information and explanations about changes.

10.3 Subject's Identification and Confidentiality

A unique number is assigned to each subject that corresponds to their clinical file number, this number will be used as subject's identification in the study, as well as in the clinical study database. Once it is confirmed that all inclusion criteria and none exclusion criteria have been met, a consecutive number in ascendant order will be assigned to subjects. For each one of these numbers, it will correspond one of the three sequences as described in section 7.3.

A unique number will be assigned to each subject following information consent has been obtained. This number will be used as subject's identification in the study, as well as in the clinical study database. All data collected from study subjects will be recorded in appropriate charts. Only the Investigator will be able to link the test's data for a subject by means of an identification list which will be located at the site. For each subject, clinical data will be available for verification purposes by the monitor, audits and regulatory inspections, but subject's confidentiality will be strictly kept.

Data protection and confidentiality will be kept during data entry, processing, submission and storage. Subjects will be informed about it and they will be requested to provide their consent for data management according to the national regulations.

The Ministry of Health, Ethics and Investigation Committee and the Investigational Committee will be the only authorized bodies for reviewing study documentation, (which includes participant subject's identity data) and documents considered confidential by clinical unit at the PPD and by the analytical unit from Merck.

10.4 Medical Insurance and Subjects' Compensation

Subjects will sign a consent which states that they will receive a compensation for their participation in the study.

The Sponsor will pay treatment (or compensation, if applicable) resulting from injuries or diseases caused by their participation in the study until their resolution as per the clinical criteria. The Sponsor will not pay injuries caused by subject's negligence, irresponsible behavior or medical reasons not related to the study.

10.5 Ethics and Investigation Committee and Investigational Committee

Before study start-up, approval from the Ethics and Investigation Committee and from the Investigational Committee has to be obtained. They should provide a list of the members who compose it, as per request from the Study Sponsor or Principal Investigator. If necessary due to amendments to the clinical trial protocol, case report form or informed consent form, a new approval should be got from both Committees.

10.6 Health Authorities

The clinical study as well as the appropriate documents will be submitted to the health authorities, according to the local and national applicable regulations.

11 Study Management

11.1 Case Report Form

Case Report Forms will be completed by authorized medical staff according to the standard operating procedure, code: PPD : GOOD DOCUMENTATION PRACTICES, with legible letter and without amendments.

Paper CRF will be fully and legibly completed, using blue ink appropriate for use in official documents. Necessary amendments or corrections should be made, dated and confirmed by the Investigator or designated personnel. Whenever corrections are made to data, original entries should remain legible, and should not be deleted or corrected. Investigator or designated personnel should state reasons of corrections of relevant data. For any missing information or comments, blanks should be voided to avoid unnecessary follow-up queries. CRFs are essential documents which should be available for regulatory inspections and submissions.

11.2 Source Document and Subject's Medical Chart

The Principal Investigator should keep a paper or electronic file (medical file, and original medical records) for each subject in the study. It should be possible to identify each subject by means of this file. This file will contain the following subject's demographic and medical information and should be as complete as possible.

- Subject's complete name, date of birth, sex, height and weight.
- Clinical history.
- Previous and concomitant medications / therapies (including changes during the study).
- Study identification; that is, subject's number provided by the clinical study Sponsor, and subject's number.
- Study recruitment dates (informed consent) and visits to the site.
- Any medical exam and pre-defined clinical findings in this clinical trial protocol.
- All adverse events.
- Date when the subject abandoned the study, including any reason for study withdrawal.

All documents which contain source data should be shown, including but not limited to, electrocardiograms and laboratory tests results. These documents should have subject's number and date of procedure. If possible, this information should be printed by the equipment used to perform the assessment or measurement. As necessary, medical assessment should be conducted; all assessments should be documented, signed and dated by the Principal Investigator.

11.3 Site's File and Master File

At the beginning of the study, the Principal Investigator will receive a master file for the site which contains all necessary study documents to be completed throughout the study and which will be updated as necessary. Master file should be available for Monitor's review, Sponsor's audits and Health Authorities' inspection during and following study. It should be securely filed for at least 15 year (or more, according to the local needs or otherwise stated by the Sponsor) following study completion. Documents to be filed include the subject's identification list and the signed informed consent by the subject. In the even the master file cannot be kept at the site anymore, the Principal Investigator should inform the Sponsor/designated person.

All subjects' source documents (medical charts) should be stored at the site as long as possible, as allowed by applicable guidelines and/or according to the ICH GCP guidelines, whatever is longer. In any case, the Principal Investigator should ensure that no destruction of medical charts is done without written approval from the Sponsor.

11.4 Quality Management

Quality assurance is performed by means of follow-up to the standard operating procedures, codes: PPD , ELABORATION OF QUALITY ASSURANCE REPORT FOR CLINICAL TRIALS; PPD , FIRST STAGE OF QUALITY CONTROL AND QUALITY ASSURANCE REVIEW DURING CLINICAL STUDIES CONDUCT; PPD , SECOND STAGE OF QUALITY CONTROL AND QUALITY ASSURANCE REVIEW DURING CLINICAL STUDIES CONDUCT; and PPD , THIRD STAGE OF QUALITY ASSURANCE REVIEW DURING CLINICAL STUDIES CONDUCT.

Quality Management follows up the conduct of the study by means of quality control monitoring for the review of compliance with the clinical trial protocol, internal standard operating procedures, and applicable guidelines according to the GCP (ICH E6R1). Discrepancies and areas of improvement identified are followed up according to the standard operating procedure, code: PPD , DISCREPANCIES IDENTIFICATION AND FOLLOW-UP, AND POTENTIAL CAUSES OF DISCREPANCIES AND CONTINUING IMPROVEMENT. All information collected is analyzed for the preparation and issue of the Quality Assurance report.

11.5 Changes to the Protocol

Trial protocol amendments can be issued by the Principal Investigator and agreed with the Sponsor or vice-versa. Substantial changes should be approved by the Ethics and Investigation Committee and by the Investigational Committee.

Administrative changes which do not affect the study will be agreed with and approved by the Sponsor, the analytical unit and by the Principal Investigator. They will also be notified to the Ethics and Investigation Committee and to the Investigational Committee.

11.6 Study Report and Publication Policy

11.6.1 Clinical Study Report

After study completion, the Principal Investigator with Sponsor's advice will prepare the clinical stage report according to the Mexican Official Standard Mexican Official Standard which states tests and procedures to prove that a drug is interchangeable. Requirements which authorized third parties should fulfill for interchangeability tests. Requirements for performing biocomparability studies. Requirements which authorized third parties, investigational sites and hospitals conducting biocomparability tests should fulfill (NOM-177-SSA1-2013) and applicable guidelines.

11.6.2 Publication

All data and results, and all intellectual property rights for data and outcomes from the study will be property of Merck, which may use data for different purposes such as, submission to government health authorities or submission to other investigators.

The Investigator, although is free to use data derived from the study for scientific purposes, should discuss any publication with Merck in advance and obtain Sponsor's written consent for the pursued publication.

The Sponsor acknowledges the Investigator's right to publish outcomes once the study is completed. Anyway, the Investigator should submit a draft of the paper or summary to be published to Merck 30 days before submitting the final version for publication.

It will be reviewed soon and approval will not be unnecessarily delayed. In case of controversies between the Sponsor and the Investigators, publication content will be discussed in order to find a satisfactory solution for both parties.

12 Deviations to the Protocol

Deviations to the trial protocol which occur during study conduct have to be documented. The Principal Investigator should informed immediately to the Study Sponsor.

When a protocol deviation occurs during the clinical stage, the Principal Investigator should assess it and consider if study subject's continuation may affect protocol's outcome. Sponsor will be informed and jointly they will make a decision about subject's continuation in the study.

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14 Appendices

- **Appendix 1: Schedule of Assessments**

Randomized, open-label trial with two parallel two-way crossover designs investigating the bioequivalence between the fixed dose combination of a metformin hydrochloride 1000 mg XR and gliclazide 30 mg MR and concomitantly administered metformin hydrochloride 1000 mg XR and gliclazide 30 mg tablets in healthy male and female subjects in fed and fasted states

Trial Day	SCR	Period 1 TD -1	TD 1														TD 2			TD 3	TD 4	TD 5	TD 6	TD 7	TD 8	EOT [13]
		wash-out	TD 8 - TD 13																							
		Period 2 TD 14	TD 15														TD16			TD17	TD18	TD19	TD20	TD21		TD22
Time Post-dose (h)			Pre-dose	0	0.5	1	2	3	4	5	6	7	8	10	12	16	24	28	32	48	72	96	120	144	168	168
Informed Consent	X																									
Hospitalization [1]		←														→										
Ambulatory Visits	X																			X	X	X	X	X	X	X
Incl./Excl. Criteria	X		X [2]																							
Medical History	X																									
Body weight, height [3]	X																									X
Virus serology (HBsAg, HBc, HCV, HIV-1/-2)	X																									
Urine drugs-of-abuse & alcohol screens [4]	X	X																								
Serum Pregnancy Testing (WOCBP) [14]	X	X																								X
Physical examination [5]	X	X																		X						X
Vital Signs [6]	X		X						X				X		X		X		X	X						X
ECG 12-lead [6]	X		X																	X						X
Safety Laboratory [14] (Hematology, Coagulation, Clinical Chemistry [15], Urinalysis)	X		X																	X						X
Blood glucose determination [16]			X				X		X						X		X		X							
HGT [17]			X				X		X				X		X		X									
Randomization [9]			X																							
IMP administration [10]				X																						
PK																										
PK			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Safety																										
Adverse Events [11]	X	X	X	←												→										
Previous medication	X	X	X																							
Concomitant medication [12]				←												→										

Group 1 fasted; Group 2 fed conditions

Footnotes

[1] Hospitalization: From TD–1 until the 48 hours post-dose for each period

[2] Subject eligibility must be checked again on TD1 prior to randomization.

[3] Height [cm] (screening) and weight [kg] [screening and End of Trial (EOT)].

[4] Urine screen for drugs of abuse (cannabinoids, amphetamines, methamphetamines, opiates, methadone, cocaine, benzodiazepines, barbiturates, tricyclic anti-depressants and phencyclidine) and alcohol. The tests can be repeated at any time at the discretion of the investigator.

[5] Complete physical examinations will be conducted at screening, TD–1, TD3, TD 21, TD 24 and EOT and abbreviated examinations at the other time points indicated. Abbreviated examinations will include: oral inspection, plus heart, lungs, abdomen, and further organs if required due to symptoms.

[6] Vital signs include systolic and diastolic blood pressure, pulse rate and oral body temperature. For vital signs and electrocardiogram (ECG) assessment, the subjects should be at rest and semi-supine for at least 10 minutes before recording and should remain resting and supine during recordings.

[9] Randomization will occur once all screening activities have been completed and the subject is deemed eligible

[10] For fasting conditions: Subjects will receive IMP (investigational medicinal product) after an overnight fast of at least 10 hours. For fed conditions: the subjects will have to start the high-fat breakfast 30 minutes prior to administration of the IMP. Subjects should eat this meal within 25 minutes or less. The IMP should be administered 30 minutes after the start of the meal. For both groups the IMP will be administered with 240 mL of water. No food will be allowed for the next 4 hours, until a standardized lunch will be served

[11] Spontaneous AEs will be continously reported; subjects will be asked specifically at pre dose, 2,4,8,12,24,32,48,72,96,120,144,168 h after each administration

[12] Subjects will be asked at pre dose, 2,4,8,12,24,32,48,72,96,120,144,168 h after each administration

[13] In the event of trial discontinuation, subjects have to undergo the EOT assessments, regardless of the reason for discontinuation (at least 7 days after administration).

[14] For female subjects who are post-menopausal for less than 2 years, post-menopausal status will be confirmed by FSH level determination (at screening only): results must be within the laboratory post-menopausal range.

[15] Blood sample collection for safety laboratory assessments will be performed after a fast of at least 10 hours (including the collection at screening).

[16] By using the clinical chemistry analyzer; for comparison purposes with HGT

[17] HGT = Hematoglucotest (bedside monitoring)

In general when multiple assessments are scheduled at same time points, the following sequence should be followed: (1) ECG recording; (2) vital sign assessment; (3) PK/Lab blood sampling and (4) meal

Appendix 2: Diet

Diet to be given to subjects following dose in each of the periods will be the same in quantity and content to that provided during the previous period.

Study breakfast: two fried eggs with butter, two bacon strips, two toasts with butter, 113 g of potato croquettes and 240 mL of whole milk (approximately 800 to 1000 calories) (150, 250, and 500-600 calories from protein, carbohydrate, and fat, respectively) (FDA, 2002, EMA 2013).

The rest of the diet (Breakfast day 2 and 16, Lunch and dinner) will be as shown in the following

Energetic Distribution			
Group	**%**	**g**	**Kcal**
Carbohydrates	55	275	1100
Proteins	15	75	300
Lipids	30	67	600
Total Kcal	----	----	2000