

Clinical Protocol

Protocol number	BD1412-62CEC
	EMR200763-003
International non-proprietary name	Metformin/Gliclazide.
Title	Randomized, Open label, Single Dose, 4 Treatment, 4 Period, Crossover design (4 x 4) trial to evaluate the Bioequivalence and Secondarily Drug - Drug Interaction of Fixed Combination of Metformin Tablets 1000 mg/Gliclazide 30 mg MR, Compared with the Co-administration of Individual Tablets and Individual Administration of Each single tablet (Metformin 1000 mg XR and Gliclazide 30 mg MR) in Healthy Volunteers.
Phase	Phase I (Bioequivalence, Drug - Drug Interaction).
IND Number	not applicable
EudraCT Number	not applicable
Principal Investigator	PPD [REDACTED]
Sponsor	Merck KGaA Darmstadt, Germany Address Frankfurter Strasse 250 64293 Darmstadt, Germany
Study Monitor	PPD [REDACTED]
Date of elaboration and version	18/May/2017, version 1.0

[REDACTED]

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Drug's name: Metformin/Glicazide
Protocol number EMR200763-003/ BD1412-62CEC

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Drug's name: Metformin/Glicazide
Protocol number EMR200763-003/ BD1412-62CEC

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Abbreviations list

A	Absorption
AE	Adverse Event
AMP	Adenosine monophosphate activated
ASA	Acetylsalicylic Acid
AUC	Area under the plasma/serum - concentration time curve
AUC_{0-∞}	Area under the plasma/serum concentration time curve from time zero to infinity
AUC_{0-t}	Area under the plasma/serum concentration time curve from time zero to the last sampling time at which the concentration is at or above LLOQ
BC	Blood Chemistry
BMI	Body Mass Index
CARPERMOR	Grupo Diagnóstico Médico PROA, S.A. de C.V.
CBC	Complete Blood Count
CI	Confidence interval
C_{max}	Maximum Plasma Concentration
CNFV	Mexican National Center of Pharmacovigilance
COFEPRIS	Federal Commission for the Protection against Sanitary Risks
CRF	Case Report Form
CV	Coefficient of variation
DBP	Diastolic Blood Pressure
dL	Deciliter
DPP4i	Inhibitors of dipeptidyl peptidase 4
E	Elimination
ECG	Electrocardiogram
EOT	End of Trial
FDA	Food and Drug Administration
FDC	Fixed-dose combination
g	Grams
GCP	Good Clinical Practice
GLP-1-RA	Glucagon like peptide-1 receptor agonists
h	Hour

HAV	Hepatitis A virus
HBs	Hepatitis B surface
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference of Harmonization
IPP	Prescribing information
ITESM	Instituto Tecnológico de Estudios Superiores de Monterrey
IUPAC	International Union of Pure and Applied Chemistry
IV	Intravenous
K_e	Elimination Rate Constant
L	Litre
mg	Milligram
min	Minute
mL	Milliliter
MR	Modified release
MRT	Mean Residence Time
ng	Nanogramm
pC	Plasma Concentration
PI	Principal Investigator (Study Coordinator, Clinical Unit)
PK	Pharmacokinetics
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SD	Standard Deviation
SGPT	Serum glutamic pyruvic transaminase
SOPs	Standard Operating Procedures
SUR1	Sulphonylureas receptor
t	Time
t^{1/2}	Elimination Half Time
t_{max}	Time of peak plasma concentration
UA	Urinalysis
WOCBP	Women of childbearing potential

1 Summary

Protocol number	EMR200763-003
Title	Randomized, Open label, Single Dose, 4 Treatment, 4 Period, Crossover design (4 x 4) trial to evaluate the Bioequivalence and Secondly Drug - Drug Interaction of Fixed Combination of Metformin Tablets 1000 mg/Gliclazide 30 mg MR, Compared with the Co-administration of Individual Tablets and Individual Administration of Each Single Tablet (Metformin 1000 mg XR and Gliclazide 30 mg MR) in Healthy Volunteers.
Phase	Phase I (Bioequivalence, Drug - Drug Interaction).
IND Number	NA
IND study	No
Number EudraCT	NA
Principal Investigator	PPD
Sponsor	Merck KGaA
Sponsor's legal representative at the European Union	Merck KGaA
Sites/Countries	Not applicable
Scheduled study period (first subject and last subject)	Not applicable
Trial Registry	COFEPRIS
Objectives:	<p>Primary objectives</p> <ul style="list-style-type: none"> • Demonstrate bioequivalence of the new fixed dose modified release combination for Metformin 1000 mg plus Gliclazide 30 mg MR compared to the concomitant administration of single tablets of Metformin 1000 mg XR and Gliclazide 30 mg MR. • Demonstrate the absence of drug interaction between the combination and the administration of each single tablet. <p>Secondary objective is Clinical safety and tolerability will also be assessed.</p>
Methodology:	A randomized, open label, single dose, 4 periods, 4 treatments crossover design will be used with 40 subjects in fasting state with a 14-day wash-out period along 4 study stages.



Planned number of subjects: 40 healthy subjects; 20 female and 20 male

Primary endpoints:

- To demonstrate bioequivalence of the fixed combination of Metformin tablets 1000 mg XR plus Gliclazide 30 mg MR compared to the co-administration of the individual tablets of Metformin 1000 mg XR and Gliclazide 30 mg MR, given as single dose to healthy volunteers in fasting state. Primary endpoints will be AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} for Metformin and Gliclazide.
- To demonstrate the lack of an effect of Gliclazide on the PK of Metformin by comparing the Metformin PK following administration of the fixed combination tablet Metformin/Gliclazide and Metformin alone (AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} of Metformin).
- To demonstrate the absence of an effect of Metformin on the PK of Gliclazide by comparing the Gliclazide PK following administration of the fixed combination tablet Metformin/Gliclazide and Gliclazide alone (AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} of Gliclazide).

Secondary endpoints:

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

Pharmacokinetic parameters:

Primary endpoints will be AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} for Metformin and Gliclazide.

Other assessments: To assess clinical safety (vital signs, ECGs, lab data, physical examinations) and tolerability for all experimental treatments through adverse event report and the effect of Metformin on Gliclazide PK and vice versa.

Diagnoses and key inclusion and exclusion criteria:

Inclusion criteria:

1. Subject has given written informed consent before any study-related activities are carried out
2. Ethnic origin: Mexicans (e.g. Caucasians, Indigenous peoples and Mestizos)
3. Age between 18 and 55 years old, inclusive.
4. Female and male subjects (at least 30% of each gender).
5. Weight between 55 and 95 kg.
6. Body mass index between 18.5 and 27 kg/m².
7. Not smoking more than 5 cigarettes or 1 cigar or 1 pipe per day (or non smokers).
8. Good physical and mental health status, determined on the basis of the medical history and physical examination.
9. Vital signs (blood pressure and pulse) in supine position within the normal range or showing no clinically relevant deviation as judged by the Investigator.
10. Electrocardiogram recording (12-lead) without signs of clinically relevant pathology in particular QTc (Bazett) <450 ms.
11. All values for biochemistry and hematology tests of blood and urine within the normal range or showing no clinically relevant deviation as judged by the Investigator.
12. All women of childbearing potential (WOCBP) are not nursing, are not pregnant, and are using highly effective methods of birth control (defined as those, alone or in combination, that result in a low failure rate (i.e., less than 1 percent per year) when used consistently and correctly) for a period of at least one month before and after dosing. Standard birth control methods are considered to be: barrier methods and intra-uterine devices. Female volunteers may also be enrolled if they are postmenopausal (i.e. at least 12 consecutive months of amenorrhea after the last menstrual period) or surgically sterilized/ hysterectomized at least 6 months prior to study participation.
13. 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 9.4.3.
14. Negative screen for alcohol and drugs of abuse (opiate class, barbiturates, cocaine and metabolites, amphetamines, cannabinoids, benzodiazepines and tricyclic antidepressants) at Screening and on each admission.
15. Negative screen for HBs antigens, HCV antibodies, HAV antibodies and HIV 1 and 2 antibodies.

Exclusion criteria:

Subjects are not eligible for this study if they fulfill one or more of the following exclusion criteria:

1. Participation in a clinical trial within 90 days prior to first drug administration.
2. Subjects who have donated more than 500 mL of blood or who have lost significantly (more than 450 mL) blood within 90 days prior to first drug of administration.
3. Any surgical or medical condition, including findings in the medical history or in the pre-study assessments, or any other significant disease, that in the opinion of the

- investigator, constitutes a risk or a contraindication for the participation of the subject in the study or that could interfere with the study objectives, conduct or evaluation.
4. History of surgery of the gastrointestinal tract which could influence the gastrointestinal absorption and/or motility according to the Investigator's opinion.
 5. Allergy: ascertained or presumptive hypersensitivity to the active drug substance and/or formulations' ingredients; history of anaphylaxis to drugs or allergic reactions in general, which the Investigator considers may affect the outcome of the trial.
 6. Receipt of any prescription or non-prescription medication within 2 weeks before the first study drug administration, including multivitamins and herbal products (e.g. St John's Wort), including ASA, and hormonal contraceptives in females.
 7. Renal failure or renal dysfunction (creatinine clearance <80 mL/min) as assessed by using the estimated measure with the Cockcroft-Gault formula.
 8. Known lack of subject compliance or inability to communicate or cooperate with the Investigator (e.g. language problem, poor mental status).
 9. Considerable diet deviations from normal nutritional patterns.
 10. Consumption of large quantities of methylxanthine-containing beverages (more than 600 mg caffeine / day: one cup (240 mL) of coffee contains approx. 100 mg of caffeine, one cup of tea ~ 30 mg and one glass of cola ~ 20 mg caffeine).
 11. Consumption of grapefruit, orange, cranberry or juices of these fruits, 14 days prior to drug administration and during the study.
 12. Legal incapacity or limited legal capacity.
 13. Subjects kept in detention.

Study drug: dosage/route/dosing schedule:

Treatment 1: dosing of fixed combination of Metformin 1000 mg and Gliclazide 30 mg MR (single oral dose of one tablet).

Reference drug: dosage/route/dosing schedule:

Treatment 2: concomitant oral dosing of one tablet of Metformin 1000 mg XR and one tablet of Gliclazide 30 mg MR

Treatment 3: single oral administration of one tablet of Metformin 1000 mg XR

Treatment 4: single oral administration of one tablet of Gliclazide 30 mg MR.

Study duration: approximately 18 weeks: eight weeks for screening phase, eight weeks approximately for conduct phase, 14 days for washout period between our periods, followed by 2 weeks of clinical follow-up.

Duration per subject: approximately 80 days (up to 30 days SCR, 3 periods a 14 days and 8 days for EOS).

Statistical methods (includes sample size calculation):

Sample size calculation

The sample size estimation is based on the CV_{intrasubject} variability of both drugs. Because the pharmacokinetic and drug-drug interaction, evaluates changes in the bioavailability, the propose is the use the same determination for both aims.

The sample size is determined by the greatest variability under fasting conditions, AUC_{0-t} of Metformin (CV=23%). Assuming a mean treatment ratio of 0.95, and aiming at a power of 90% for the individual test, alpha=0.05 one-sided, 32 evaluable subjects are needed to demonstrate bioequivalence.

The same arguments can be applied to evaluate the absence of an effect of Gliclazide on the PK of Metformin, whereas the power for the third test is close to 100% with 32 subjects, due to the low variability of Gliclazide PK. Therefore, the overall power is still greater than 80% with 32 evaluable subjects. Considering a drop-out rate of approximately 20%, 40 subjects should be randomized.

Analysis of PK parameters

Bioequivalence test methodology will be used in all 3 steps:

Bioequivalence in step1:

A mixed model will be applied to log-transformed C_{max}, AUC_{0-t} and AUC_{0-∞} (both analytes) with treatment, period and sequence as fixed effects, and subject (sequence) as a random effect. Based on the residual error term 90% confidence intervals will be computed for the estimated differences Test – Reference, resulting in 90% confidence intervals for the Test/Reference ratios after back-transformation.

Drug-rug interaction in steps 2 and 3:

A mixed model will be applied to log-transformed C_{max}, AUC_{0-t} and AUC_{0-∞} (one analyte) with treatment as fixed effect, and subject as a random effect. Based on the residual error term 90% confidence intervals will be computed for the estimated differences Test – Reference, resulting in 90% confidence intervals for the Test/Reference ratios after back-transformation.

1. Bioequivalence will be concluded, if all six 90% CIs for all six primary endpoints reside within the acceptance range [0.80 – 1.25]
2. Absence of an effect of Gliclazide on the PK of Metformin will be concluded, if all three 90% CIs for Metformin endpoints reside within the range [0.80 – 1.25]
3. Absence of an effect of Metformin on the PK of Gliclazide will be concluded, if all three 90% CIs for Gliclazide endpoints reside within the range [0.80 – 1.25]

Additionally, the primary endpoints will be descriptively analyzed and graphical displays (Box-whiskers-plots) will be prepared.

All other PK endpoints will be analyzed using descriptive methods. Graphical displays will be also prepared where appropriate (Concentration-time profiles individual and average will be display for each drug).

Analysis of safety parameters

All safety parameters will be analyzed descriptively.

2 Sponsor, Investigators and Study Administrative Staff

The study will be sponsored by:

- Merck KGaA. Address Frankfurter Strasse 250 64293 Darmstadt, Germany

The study will be conducted in one single site, PPD [REDACTED].

Principal Investigator, PPD [REDACTED] from the PPD [REDACTED], 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. She is responsible for review and approval of the clinical study report.

This protocol meets requirements of current clinical research regulations, including: ICH GCP, 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 [REDACTED]

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 the 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 protocol is justified.

3.1 Metformin's Pharmacology

3.1.1 Metformin's Chemical Structure

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

Metformin empiric formula is: C₄H₁₁N₅. Metformin's molecular weight is 129.164 g/mol and Metformin hydrochloride is 165.63 g/mol (Pubchem, 2015.)

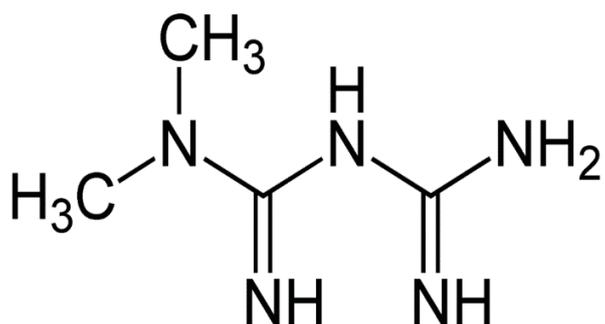


Figure 1. – Metformin

3.1.2 Absorption

Metformin's apparent distribution volume (V/F) following oral single dose of Metformin hydrochloride 850 mg tablets averaged 654 ± 358 L. Metformin is negligibly bound to plasma proteins in contrast to sulphonylureas which are more than 90 % to protein bound. Erythrocytes seem to represent the secondary distribution compartment. At usual clinical doses of Metformin hydrochloride, plasma concentration in steady state is achieved within 24 - 48 h and generally are < 1 mg/mL (Green and Feinglos, 2008, Cho, 2002). After an oral dose of Metformin XR 500 mg tablet, the T_{max} was 7 hours and C_{max} 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 is between 63 a 276 L.

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

3.1.4 Metabolism

Metformin does not undergo liver metabolism; 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 renal clearance is > 400 mL/min, which shows that Metformin is cleared by glomerular filtration and tubular secretion. Following oral dosing, elimination half-life is approximately 6.5 h (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 et al., 2005; Hundal and Inzuchi, 2003; Ristic et al., 2007).

3.1.7 Pharmacological Properties

Throughout Metformin's chronic treatment, vitamin B12, folates and calcium supplements intestinal absorption revert 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 reaction may occur with Metformin treatment: taste impairment (dysgeusia), nausea, vomiting, diarrhea, anorexia, abdominal pain and loss of appetite (CCSI V7 for Metformin hydrochloride).

The following definitions apply to the frequency terminology used hereafter: Very common ($\geq 1/10$); Common ($\geq 1/100, < 1/10$); Uncommon ($\geq 1/1,000, < 1/100$); Rare ($\geq 1/10,000, < 1/1,000$); Very rare ($< 1/10,000$).

Gastrointestinal disorders

Very common: 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

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

Metabolism and nutrition disorders

Very rare: Lactic acidosis.

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

Hepatobiliary disorders

Very rare: 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).

Gliclazide's empiric formula is: C₁₅H₂₁N₃O₃S. Gliclazide's molecular weight is 323.412 g/mol.

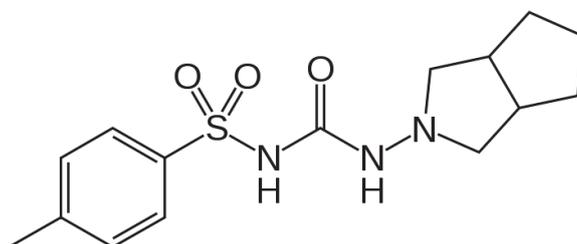


Figure 2. - Gliclazide

3.2.2 Absorption

It is quickly absorbed. After oral administration of Gliclazide, plasma concentrations increase progressively until the 6th hour, reaching plateau between the 6th and 12th hour (IPP Diamicon MR, 2013). Gliclazide is fully absorbed.

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 the placental barrier and travels in the fetal blood stream. A low apparent distribution volume is likely seen in the high binding degree to proteins (approximately 94 % of a plasma concentration of 8 mcg/mL) (Sarkar et al., 2011).

3.2.4 Metabolism

Gliclazide 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, enzymes involved 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 until 120 h following dosing (Ings et al., 1986).

Fecal clearance is approximately 11 % of the administered dose (Ings et al., 1986). Gliclazide is generally fully cleared 144 h following the last dose. (Sarkar et al., 2011). Gliclazide's elimination half-life ranges between 12 and 20 hours (IPP Diamicon 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 hypoglycemic sulphonylurea, oral anti-diabetic drug which has a nitrogen heterocyclic ring which differentiate it from other drugs of this type.

It stimulates insulin secretion from islets of Langerhans β cells. Increase of postprandial insulin secretion and C-peptide persists 2 years following therapy (Ristic et al., 2007).

Besides its metabolic properties, Gliclazide has hemovascular properties.

- Effect on the insulin release: In type 2 diabetes patients, Gliclazide repairs early peak of insulin secretion upon presence of glucose and increases the second phase of that secretion. There is a significant increase in the insulin's response following stimulation induced by food or glucose.
- Hemovascular properties: Gliclazide reduces microthrombosis process by means of two mechanisms which might be implied in Diabetes complications:
 - a) Partial inhibition of platelet aggregation and adhesion with a reduction of platelet activation markers (beta-thromboglobulin, thromboxane B2).
 - b) An effect on a vascular endothelium's fibrinolytic activity with an increase of the t-PA activity.

3.2.8 Adverse reactions

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.

Gastrointestinal disorders have been reported, including abdominal pain, nausea, vomit, dyspepsia, diarrhea and constipation; these might be avoided if tablet is taken in fasting state.

The following adverse reaction have been reported less frequently:

- Skin rash - mucosal sore: rash, pruritus, hives, maculopapular rash, and blisters.
- Blood disorders: they are rare, anemia, leukopenia, thrombocytopenia, and granulocytopenia. These are generally reverted upon therapy stop.
- Hepatobiliary disorders: liver enzymes elevation (AST [SGOT], ALT [SGPT], and alkaline phosphatase), or hepatitis (isolated cases.) Therapy should be interrupted if cholestatic jaundice occurs. These symptoms usually disappear following treatment stop.

• Eye disorders: visual transient discomforts may occur specially at the beginning of treatment due to glycemetic changes.

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

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

Hypoglycaemia

As for other sulphonylureas, Gliclazide can cause hypoglycaemia, if mealtimes are irregular and, in particular, if meals are skipped. Possible symptoms of hypoglycaemia 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 hypoglycaemia can recur even when measures prove effective initially. If a hypoglycaemic episode is severe or prolonged, and even if it is temporarily controlled by intake of sugar, immediate medical treatment or even hospitalisation is required.

The following definitions apply to the frequency terminology used hereafter: Very common ($\geq 1/10$); Common ($\geq 1/100, < 1/10$); Uncommon ($\geq 1/1,000, < 1/100$); Rare ($\geq 1/10,000, < 1/1,000$); Very rare ($< 1/10,000$).

Blood and lymphatic system disorders

Rare: Hematologic disturbances which may include anaemia, 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 the fixed dose combination (Metformin/Gliclazide). Consideration of such etiology is recommended if a patient presents with megaloblastic anaemia.

Frequency not known: Hypoglycaemia.

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, diarrhoea, 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 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 urticaria.

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 the fixed dose combination of Metformin and Gliclazide belongs to sulphonylureas, the following adverse events have been observed: cases of erythrocytopenia, agranulocytosis, haemolytic anaemia, pancytopenia, allergic vasculitis, hyponatremia.

5 Study Objectives

5.1 Primary Objective

- ✚ To demonstrate the bioequivalence of Metformin/Gliclazide fixed dose combination tablet (1000 mg /30 mg MR) compared with the co-administration of the individual tablets (Metformin 1000 mg XR and Gliclazide 30 mg MR), administered as single dose in fasting condition to healthy volunteers.
- ✚ To demonstrate the absence of an effect of Gliclazide on the PK of Metformin by comparing the Metformin PK following administration of the fixed combination tablet Metformin/Gliclazide and Metformin alone (AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} of Metformin).
- ✚ To demonstrate the absence of an effect of Metformin on the PK of Gliclazide by comparing the Gliclazide PK following administration of the fixed combination tablet Metformin/Gliclazide and Gliclazide alone (AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} of Gliclazide).

5.2 Secondary Objectives:

- ✚ To assess clinical safety and tolerability for all experimental treatments.

6 Statistical Hypothesis

1. Test bioequivalence of fixed combination and separate tablets

$H_0: \mu_T / \mu_R \leq 0.8$ or $1.25 \leq \mu_T / \mu_R$ for Metformin and/or Gliclazide AUC_{0-t} , $AUC_{0-\infty}$ or C_{max} ,
or for all

$H_1: 0.8 < \mu_T / \mu_R < 1.25$ for all Metformin and Gliclazide primary endpoints (i.e. AUC_{0-t} ,
 $AUC_{0-\infty}$ or C_{max})

μ_T and μ_R being the means under Test and Reference treatment, respectively.

Test treatment: Metformin/Gliclazide fixed dose combination tablet (1000 mg /30 mg MR).

Reference treatment: co-administration of the individual tablets (Metformin 1000 mg XR and Gliclazide 30 mg MR).

Bioequivalence is confirmed if all six 90% CIs for all six primary endpoints reside within the acceptance range [0.80 – 1.25].

2. Test absence of an effect of Gliclazide on the PK of Metformin

$H_0: \mu_T / \mu_R \leq 0.8$ or $1.25 \leq \mu_T / \mu_R$ for Metformin AUC_{0-t} , $AUC_{0-\infty}$ or C_{max} , or for all

$H_1: 0.8 < \mu_T / \mu_R < 1.25$ for all Metformin primary endpoints (i.e. AUC_{0-t} , $AUC_{0-\infty}$ or C_{max})

μ_T and μ_R being the means under Test and Reference treatment, respectively.

Test treatment: Metformin/Gliclazide fixed dose combination tablet (1000 mg /30 mg MR).

Reference treatment: Single administration of Metformin 1000 mg XR

Absence of an effect of Gliclazide on the PK of Metformin will be concluded, if all three 90% CIs for Metformin endpoints reside within the range [0.80 – 1.25]

3. Test absence of an effect of Metformin on the PK of Gliclazide

$H_0: \mu_T / \mu_R \leq 0.8$ or $1.25 \leq \mu_T / \mu_R$ for Gliclazide AUC_{0-t} , $AUC_{0-\infty}$ or C_{max} , or for all

$H_1: 0.8 < \mu_T / \mu_R < 1.25$ for all Gliclazide primary endpoints (i.e. AUC_{0-t} , $AUC_{0-\infty}$ or C_{max})

μ_T and μ_R being the means under Test and Reference treatment, respectively.

Test treatment: Metformin/Gliclazide fixed dose combination tablet (1000 mg /30 mg MR).

Reference treatment: single administration of Gliclazide 30 mg MR

Absence of an effect of Metformin on the PK of Gliclazide will be concluded, if all three 90% CIs for Gliclazide endpoints reside within the range [0.80 – 1.25]

7 Investigational Plan

7.1 Experimental design and study plan

This randomized, open-label, single dose, four-treatment, four period crossover design (4x4) trial with a 14-day wash-out period along 4 study stages will investigate the bioequivalence (primary endpoints are AUC_{0-t} , $AUC_{0-\infty}$ or C_{max} for Metformin and Gliclazide) of a Metformin/Gliclazide fixed combination tablet (1000 mg /30 mg MR) compared with the co-administration of the individual tablets (Metformin 1000 mg and Gliclazide 30 mg MR) and the drug-drug interaction between the Metformin/Gliclazide fixed combination and the administration of each single tablet, administered in fasted condition in 40 healthy volunteers. Secondly, safety and tolerability will be also investigated in every case.

The subjects will be randomized to one of the 4 treatment sequences (see table below). Ten subjects will be randomized to each treatment sequence with a minimum proportion of 30% for each sex in each treatment sequence.

Sequence	Period 1	Wash-out period	Period 2	Wash-out period	Period 3	Wash-out period	Period 4
1	Fixed combination		Metformin + Gliclazide		Metformin		Gliclazide
2	Metformin + Gliclazide		Gliclazide		Fixed combination		Metformin
3	Metformin		Fixed combination		Gliclazide		Metformin + Gliclazide
4	Gliclazide		Metformin		Metformin + Gliclazide		Fixed combination

7.2 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 hemoglobin A1c under 7% as target for more than 3 months, then it will be necessary to add a second oral agent from another pharmacological family, such as 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's and Gliclazide's association and it is particularly recommended for some clinical guidelines (Treatment Algorithm for Type 2 Diabetes, 2014) when Metformin's 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 satisfactory benefit/risk.

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.)

In case of combinations which contain the active ingredients (as proposed in this study) and with a replacement indication (i.e., use in adequately controlled patients with individual products administered simultaneously, at the same dosage level than in the combination, but in different tablets), bioequivalence should be proven between free formulations combination and marketing formulation (fixed combination) (EMA, 2009).

On the other hand, international guidelines recommend that in case of new fixed combinations, it is necessary to assess the degree which drugs affect mutually the appropriate pharmacokinetic pattern (drug - drug interaction) (EMA, 2009).

Likewise, in Mexico, the committee to assess the marketing approval for new molecules (New Molecules' Committee) in case of new fixed dose combinations requests evidence about the lack of interaction between compounds making up the combination (drug - drug interaction) (COFEPRIS, 2013).

Clinical trial herein proposed is designed to achieve objectives stated by international guidelines as Mexico's Health Authorities in the field of bioequivalence and interactions. Although both objectives could be studied in two separate clinical trials (bioequivalence crossover design 2 x 2 and a cross interaction design 3 x 3), it is deemed more efficient to combine them in a single study. This reduces the number of subjects exposed as well as the minimization of time and costs. Therefore, the purpose of this clinical trial is mainly demonstrating bioequivalence and the drug-drug interaction of the new fixed dose combination for Metformin 1000 mg plus Gliclazide 30 mg MR compared to the concomitant dosage of individual tablets of Metformin 1000 mg XR and Gliclazide 30 mg MR. As secondary objective clinical safety and tolerability for all treatments used will be assessed.

7.3 Study population screening

Only those subjects who meet all inclusion criteria and none of the 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 10.2, Subject's information and informed consent form.

- ✚ Recruitment by invitation by means of an open announcement.
- ✚ Inclusion of 40 subjects in study as per protocol's criteria.
- ✚ Female and male subjects (at least 30% of each gender per treatment sequence).

7.3.1 Inclusion criteria:

For study inclusion, all of the following criteria must be fulfilled:

1. Subject has given written informed consent before any study-related activities are carried out
2. Ethnicity origin: Mexicans (e.g., Caucasian, Indigenous people and mestizos [mixed race])
3. Gender: male and female (at least 30% of each gender)
4. Age between 18 and 55 years old, inclusive.
5. Weight between 55 and 95 kg.

6. Body mass index between 18.5 and 27 kg/m².
7. Not smoking more than 5 cigarettes or 1 cigar or 1 pipe per day (or non smokers).
8. Good physical and mental health, determined on the basis of the medical history and a physical examination.
9. All results from blood chemistry, hematology, and urinalysis should be within normal ranges or without clinically significant deviations as per Principal Investigator's judgment.
 - ✓ 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 \pm 15 %, if subject is asymptomatic.
 - ✓ Liver Function Test (LFT), including: direct and indirect bilirubin, glutamic oxaloacetic transaminase (aspartate aminotransferase) and SGPT (ALT), 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.
 - ✓ Blood chemistry (BC) including at least: glucose, urea, creatinine and uric acid. Asymptomatic subjects without family history of Diabetes or renal failure may be included with values ranging \pm 15 %.
 - ✓ Lipids panel including total cholesterol and triglycerides. Asymptomatic subjects without family history of dyslipidemia may be included with values ranging \pm 15 %. Lower limit does not have restriction for subject's inclusion.
 - ✓ Urinalysis (UA): asymptomatic subjects without history of renal failure may be included with values of urinary density ranging \pm 1 %. pH should be within 5 and 7. They can be included with albumin trace.
10. Electrocardiogram recording (12-lead) without signs of clinically relevant pathology in particular QTc (Bazett) <450 ms.
11. Vital signs (blood pressure and pulse) in supine position within normal ranges or showing no clinically relevant deviation as judged by the medical Investigator.
 - ✓ Heart rate between 50 and 100 beats per minute.
 - ✓ Respiratory rate between 12 and 20 per minute.
 - ✓ Systolic blood pressure between 80 and 129 mmHg.
 - ✓ Diastolic blood pressure between 50 and 89 mmHg.
 - ✓ Temperature between 36.0 and 37.0 °C.

12. All women of child bearing 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.
13. 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 9.4.3
14. Negative result for oral alcohol screening at the beginning of each visit (day -1 for treatment period) and illegal drugs abuse (opioids, barbiturates, cocaine and its metabolites, amphetamines, metamphetamines, morphine, cannabinoids, benzodiazepines and tricyclic antidepressants), throughout the screening period and start of each one of the visits (day -1 for each treatment period).
15. Serology tests negative for human immunodeficiency virus (HIV1 and HIV2 antibodies), hepatitis A (HAV), hepatitis B (HBV), hepatitis C (HCV) and VDRL test screening. Valid for 3 months.

7.3.2 Exclusion criteria:

1. Participation in a clinical trial within 90 days prior to first drug administration.
2. Subjects who have donated more than 500 mL of blood or who have lost significantly (more than 450 mL) blood within 90 days prior to first drug of administration.
3. Any surgical or medical condition, including findings in the medical history or in the pre-study assessments, or any other significant disease, that in the opinion of the investigator, constitutes a risk or a contraindication for the participation of the subject in the study or that could interfere with the study objectives, conduct or evaluation.
4. History of surgery of the gastrointestinal tract which could influence the gastrointestinal absorption and/or motility according to the Investigator's opinion.
5. Allergy: ascertained or presumptive hypersensitivity to the active drug substance and/or formulations' ingredients; history of anaphylaxis to drugs or allergic reactions in general, which the Investigator considers may affect the outcome of the trial.
6. Receipt of any prescription or non-prescription medication within 2 weeks before the first study drug administration, including multivitamins and herbal products (e.g. St John's Wort), including ASA, and hormonal contraceptives in females.
7. Renal failure or renal dysfunction (creatinine clearance <80 mL/min) as assessed by using the estimated measure with the Cockcroft-Gault formula.

8. Known lack of subject compliance or inability to communicate or cooperate with the Investigator (e.g. language problem, poor mental status).
9. Considerable diet deviations from normal nutritional patterns.
10. Consumption of large quantities of methylxanthine-containing beverages (more than 600 mg caffeine / day: one cup (240 mL) of coffee contains approx. 100 mg of caffeine, one cup of tea ~ 30 mg and one glass of cola ~ 20 mg caffeine).
11. Consumption of grapefruit, orange, cranberry or juices of these fruits, 14 days prior to drug administration and during the study.
12. Legal incapacity or limited legal capacity.
13. Subjects kept in detention.

7.3.3 Criteria for Randomization

Eligible subjects will be rechecked at the time of admission to the study center (Day -1).

Results of the procedures performed on Day -1 as detailed Section 9.1.1, must be available prior to randomization. All inclusion and exclusion criteria will undergo a final verification before randomization.

7.3.4 Criteria for Subject Withdrawal from trial:

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

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

1. Hypertension signs and symptoms which require pharmacological treatment.
2. Syncope or shock.
3. Severe adverse reaction.
4. Serious Adverse Event.
5. Concurrent diseases or concomitant treatments not allowed by the protocol.
6. Lack of discipline.
7. Lack of compliance with the diet.
8. Lost of two samples within absorption period or near to the C_{max} .
9. If vomit occurs within therapeutic dosing interval, research subject should be withdrawn from the study and removed from the statistical analysis.

10. If any of the participant subject, during wash-out period, takes alcoholic beverages, smokes tobacco, has xanthenes 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.
11. Subjects with positive results to the drugs screening such as: amphetamines, benzodiazepines, cocaine, metamphetamines, morphine and tetrahydrocannabinoids.
12. Positive pregnancy test (qualitative or quantitative) following the study drug dosing.
13. All cases where the Principal Investigator judges as lack of compliance to the protocol or which may risk final results.

7.4 Start-up Criteria for 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 must have negative result for oral alcohol screening at SCR and at the beginning of each visit (day -1 for treatment period) and drugs of abuse (opioids, barbiturates, cocaine and its metabolites, amphetamines, metamphetamines, morphine, cannabinoids, benzodiazepines and tricyclic antidepressants), throughout the screening period and start of each one of the visits (day -1 for each treatment period).

7.5 Study Subject Withdrawal

Participant subjects in the study are free to withdraw from the study at any time and they will only be asked to give the reason of their withdrawal. An end of study examination must be performed if the subject has received at least one active drug as describe in section 8.2.7. Withdrawal reason should be documented in the medical chart in the case report form, if known. Study drug assigned to the dropped subject cannot be assigned to another subject. Subjects who withdraw after at least one IMP administration will not be replaced.

7.5.1 Withdrawal from the study

A subject must be withdrawn from the study for any event which might risk subject's safety. In the event pregnancy tests during subjects' admission are positive, their participation will be stopped immediately (for further procedure in case of pregnancy see section 8.5.2).

A subject should be withdrawn from the study in case of any of the following:

- Voluntary subject's withdrawal.
- Lost to follow-up.
- Participation in another investigational study.

7.6 Study termination

The clinical study can be finished or stopped early 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 schedule 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.

7.7 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).

8 Investigational Drug and Other Drugs Used in the Study

1. Test drug: Fixed combination for Metformin/Gliclazide.
2. Reference drug: Concomitant dosing of Metformin + Gliclazide
3. Reference drug: Dosing of Metformin alone.
4. Reference drug: Dosing of Gliclazide alone.

8.1 Investigational Drug Description

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

- + International non-proprietary name: Metformin/Gliclazide.
- + Reference drug: Dabex[®] XR.
 - Dosage form: tablets
 - Quali-Quantitative Formula: each tablet contains 1000 mg of Metformin hydrochloride.
 - Manufacturer: Merck KGaA
 - Distributor: MERCK, S. A. de C. V.
 - Marketing Holder: MERCK, S. A. de C. V.
- + Reference drug: Diamicon MR[®].
 - Dosage form: extended release tablets
 - Quali-Quantitative Formula: Each tablet contains 30 mg of Gliclazide.
 - Manufacturer: Les Laboratoires Servier Industrie
 - Distributor: Beckman Laboratories de México, S. A. de C. V.
 - Marketing Holder: Beckman Laboratories de México, S. A. de C. V.
- + 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 MR.
 - Treatment 1: dosing of fixed combination of Metformin 1000 mg/Gliclazide 30 mg MR (one tablet).

Drug's name: Metformin/Gliclazide
Protocol number EMR200763-003/ BD1412-62CEC

- Treatment 2: concomitant dosing of Metformin 1000 mg/Gliclazide 30 mg (two tablet.)
- Treatment 3: dosing of Metformin 1000 mg (one tablet.)
- Treatment 4: dosing of 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.

Quantity required for each stage of the research: 11 tablets.

60 tablets for the study.

220 tablets for the finished product analysis and dissolution profile:

60 tablets as retention sample.

Manufacturer: Merck KGaA

Products importer:

Merck S.A. de C.V.

PPD 

PPD 

Merck LATAM

PPD 





8.2 Dosage and administration

After 10 hours of fasting conditions the dose to be used in the study will be the fixed Metformin XR 1000 mg/Gliclazide MR 30 mg combination as single dose of the test product or the individual reference products as appropriate for each period.

8.3 Randomization to treatment groups

Once it is confirmed that all inclusion criteria and none exclusion criteria have been met, subjects will be randomized to one of four treatment sequences. Randomization of each subject to a treatment sequence will occur prior to dosing on Day 1. The Investigator or delegate will allocate from the treatment kit stock (numbered 101-140 the next available treatment kit in a sequential, chronological order starting with the lowest number. Randomization will be based on a unique randomization list.

The Principal Investigator will perform the randomization list before the Day 1, the list will be generated from the Web Site Randomization.com (<http://www.randomization.com>). Once obtained, the format PPD [REDACTED] "Treatment Sequence" will be developed which will review Quality Management according to the standard operation procedure, code: PPD [REDACTED], randomizing a clinical study of interchangeability and comparability

An additional set of 12 treatment kits for possible replacers will be provided. There will be 3 treatment kits per treatment sequence. Replacement kit numbers will be 987 to 999. The pharmacist will assign the open-label replacement kit according to the treatment sequence of the originally assigned kit.

8.4 Selection and Dosing Schedule for Dosing for Each Subject

Forty male and female subjects will be included in this trial. Both sexes will be included with a minimum proportion of 30% for each sex at least 12 females and at least 12 males.

Ten subjects will be randomized to each treatment sequence with a minimum proportion of 30% for each sex in each treatment sequence.

The administration of the test or reference drug will be held at morning (about 08:00 h) after the fasting period (minimum eight hours) and follow the randomization list. It shall be recorded in the Case Report Form the current time of drug administration

During each period one of the four treatments will be dosed for all subjects from 08:00 till 08:14 h, in 8 groups of 5 subjects, with a 2-minute gap among each group for dosing and each one of the activities. Group's distribution can be changed due to operational reasons, as long as there is no impact in the study results.

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

Dosing scheme

Group	No. of subjects	Dosing schedule
A	01, 02, 03, 04 and 05	08:00 h
B	06, 07, 08, 09 and 10	8:02 h
C	11, 12, 13, 14 and 15	8:04 h
D	16, 17, 18, 19 and 20	8:06 h
E	21, 22, 23, 24 and 25	8:08 h
F	26, 27, 28, 29 and 30	8:10 h
G	31, 32, 33, 34 and 35	8:12 h
H	36, 37, 38, 39 and 40	8:14 h

8.5 Concomitant therapies and drugs

Subjects are not allowed to take any drug within 7 half-lives (14 days) prior to the study drug dose. This restriction should be followed during the 4 study periods and the wash-out periods among doses.

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

8.5.1 Allowed Drugs

Concomitant use of drugs is not allowed, unless those prescribed for adverse events and to protect subjects' wellbeing who will be allowed to continue in the study as per the Principal Investigator's judgment. This should be justified in the medical chart.

8.5.2 Forbidden Drugs

Subjects who take any other drug, prescribed or over the counter drugs 2 weeks 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) in addition to ASA and oral contraceptives for women.

8.5.3 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 to participate in the study.

8.5.4 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 duty 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.

8.5.5 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 Principal Investigator will decide the subsequent management, according to the standard operating procedure, code: PPD Management of clinical eventualities.

8.6 Investigational Drug Packaging and Labelling

Diabex XR[®] de Merck, S.A. de C.V., México

Reference drug will be provided by the Sponsor, in commercial dosage form (original primary and secondary packaging).

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 product's needs.

Reference product will be given orally at a dose of 1000 mg (one tablet) of Metformin XR, given with 250 mL of water. Single dose.

Diamicron[®] MR, Laboratorios Servier México

Reference drug will be provided by the Sponsor, in commercial dosage form (original primary and secondary packaging 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 product's needs.

Drug's name: Metformin/Glicazide
Protocol number EMR200763-003/ BD1412-62CEC

Reference product will be given orally at a dose of 30 mg (one table) of Gliclazide XR, given with 250 mL of water. Single dose.

Fixed dose combination of MR Metformin 1000 mg and Gliclazide 30 mg from Merck, S.A., Brazil

Reference drug will be provided by the Sponsor, in commercial dosage form (original primary and secondary packaging 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 product's needs.

Test product will be given orally at a dose of Metformin 1000 mg/Gliclazide 30 mg (one tablet) with 250 mL of water. Single dose.

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

8.7 Preparation, Management and Storage of Study Drug

Test and reference drugs will be received at the PPD [REDACTED] by the Pharmacy Responsible or by the designated staff, as per the standard operating procedures, code: PPD [REDACTED] Receipt, use, balance and final disposition of the study drug. Once drugs have been identified, they will be kept by the clinical unit until they are administered to the subjects.

Acceptance criteria for test and reference drugs.

- ✚ Complete documentation:
 - ✓ Certificate of Analysis.
 - Dosage units.
 - Content assessment.
 - ✓ Letter of Good Manufacturing Practice compliance for test and reference drug.
 - ✓ Copy of the invoice for the reference drug.
- ✚ Test drug with label compliant with the minimum requirements from NOM-072-SSA1-2012, Labelling of Drugs and Herbal Remedies.
- ✚ Enough quantity of test and reference drug.

- ✚ Expiry date not due at the moment of use in the clinical study.
- ✚ Test and reference drug in good physical conditions.

Rejection criteria for test and reference drugs.

- ✚ Blister 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 according to the label information.

8.8 Study Drug Accountability

The Sponsor will ship enough quantities of the test and reference product to conduct the study and keep some as "retention samples" to the PPD [REDACTED]

The Principal Investigator, Pharmacy Responsible or designated staff at the PPD [REDACTED] 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 [REDACTED]. Receipt, use, balance and final disposition of the study drug.

8.9 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 [REDACTED]. Preparation and administration of drugs in clinical trials of interchangeability and biocomparability; PPD [REDACTED]. Randomization in an interchangeability and biocomparability clinical study

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

8.10 Method of Blinding

Not applicable, this is an open-label trial.

8.11 Emergency Unblinding

Not applicable, this is an open-label trial.

8.12 Overdose treatment

Overdose is defined as any dose higher than the highest daily dose included in the clinical 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 [REDACTED] 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 8.5.1. If the overdose caused a SAE, it has to be reported immediately the sponsor according to the section 10.1.1.2.

The study medication will be prepared and administered under the supervision of the Principal Investigator, once administered the study drug, the drug will secure again in the area of pharmacy, which will be restricted for access.

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 given 10 mL of concentrated glucose solution (20 to 30 %) within 1 or 2 hours following the dose. Glucose solution charges are not limited to these two occasions. If hypoglycemia symptoms occur, additional glucose solution can be given. The concentrated glucose solution (20 to 30 %) 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:

Hypoglycaemia 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 haemodialysis.

Symptoms and Management of overdose of the combination drug product (Metformin and 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 hypoglycaemia.

Management

Management of a Metformin overdose:

The most effective method to remove lactate and Metformin is haemodialysis.

Management of a Gliclazide overdose:

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

If hypoglycaemia coma is diagnosed or suspected, the patient should be given a rapid i.v. injection of 50 mL of concentrated glucose solution (20 to 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.

Hypoglycaemia 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 haemodialysis (CCDS Metformin V7).

8.13 Medical Care for Subjects Following the End of the Study

Subjects will sign a consent, which states that they will receive an economical 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 no related to 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.

9 Study Assessments and Procedures

9.1.1 Subjects screening

Screening of the subjects will take place within 30 days prior to the first administration of the trial medication and comprises the following:

- Informed consent for participation in the trial,
- Inclusion/exclusion criteria checked.
- Demographics (including nicotine consumption, smoking history, alcohol consumption, intake of caffeine or xanthine-containing beverages, intake of grapefruit, orange, cranberry or juices of these three fruits and diet as described in Section 6.3.2)
- Medical history and history of medications
- Vital signs (body temperature [tympanic], as well as blood pressure (BP) and pulse rate after at least 5 minutes rest in supine position),
- Physical examination (including measurement of weight and height),
- Safety laboratory: biochemistry tests, hematological tests and urinalysis, coagulation and virology,
- A urine drug screen and alcohol breath test,
- Serum pregnancy test (WOCBP only),
- 12-lead standard ECG (after 5 minutes rest in supine position),

AEs, concomitant medications and procedures will be continuously assessed from signature of informed consent to the end-of-trial examination.

If no clinically relevant finding is made at screening and the subject satisfies all of the protocol inclusion criteria and none of the exclusion criteria, he/she will be considered as eligible and will be enrolled into the clinical trial.

Subjects who fail to meet the protocol specified criteria for dosing or who withdraw their consent in the screening period are considered screening failures. The following data will be recorded for these subjects: demographics (including age, sex, weight and height), AEs, concomitant medications and reason for screening failure.

9.1.2 Study periods

At the beginning of each period, a clinical assessment consisting of an examination by apparatus and systems will be made in order to check if the subject remains eligible for the study. Blood will be taken for pregnancy tests for women of childbearing potential and urine samples for drug screening. Oral alcohol 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 care 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 each 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 from subjects for safety laboratory tests such as hematology, blood chemistry, prothrombin time (PT) and partial thromboplastin time (PTT) and urinalysis. Samples will be taken pre-dose (following 10-h fasting) and at discharge (48 h post-dose).

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

At the end of each period and at the last outpatient sample (sample 168.00 h post-dose) a clinical assessment will be made. It will consist of an interview, physical examination (oral cavity examination, lungs, heart, abdomen and other organs, if necessary in case of symptoms), vital signs measurement and follow-up of adverse events. Blood and urine samples will be taken for safety laboratory tests such as hematology; blood chemistry, 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 will be performed consisting of an interview, physical examination (oral cavity examination, lungs, heart, abdomen and other organs, if necessary in case of symptoms).

Weight and height, and vital signs will be measured; and electrocardiogram and adverse events follow-up will be carried out. Blood samples will be taken for pregnancy tests for women of childbearing potential.

For post-menopausal women (amenorrhoeic for at least one year), post-menopausal condition will be confirmed by means of follicle-stimulating hormone level 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.

9.1.3 Control of Meals and Liquid Intake

During study conduct, subjects should fast for at least 10 hours before the dosing and at least for 4 hours following the dosing. Water is allowed *ad libitum*, except for the period between one hour prior to dosing and the first meal.

During 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.

Feeding time

MEALS	DAY 0, 14, 28 and 42	DAY 1, 15, 29 and 43
Breakfast	-----	As from 12:00 h
Lunch	-----	As from 16:00 h
Dinner	21:00 h	As from 21:00 h

Groups will be outdated by two 2 minutes between each (after dosing) for food delivery for the order and interval of drug administration. Food and Liquids that were not intake completely must be described as protocol deviation.

9.2 Schedule of Assessments

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

9.2.1 First period of treatment

Day- 1:

- ✚ Admission the day prior to the visit for assuring 10-h fasting period.
- ✚ Dinner at least 10 h before the dosing.
- ✚ Subject's eligibility will be checked again before catheter insertion and drug's dosing.
- ✚ Catheter insertion (by choice, only women).

Day 1:

- ✚ At 5:00 h, grooming and intake of 250 mL of water.
- ✚ Between 05:20 and 06:30 h, electrocardiogram will be performed.
- ✚ Post electrocardiogram the following activities will be done
 - Catheter insertion (control or "0" sampling).
 - vital signs will be measured
 - Safety laboratory tests.
 - Blood glucose determination
 - Finger stick glucose
 - Adverse events and previous medication
- ✚ Randomization for the first period of treatment only
- ✚ At 08:00 h, oral administration of test or reference drug 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 sampling schedule.
- ✚ At 8:00 h, 0.5 PK sampling
- ✚ At 9:00 h, 1 PK sampling
 - At 10:00 h, 2 PK sampling and Finger stick glucose
- ✚ At 11:00 h, 3 PK sampling

- ✚ At 12:00 h, 4 PK sampling and Finger stick glucose, Blood glucose determination and vital signs
- ✚ As from 12:00 h, breakfast.
- ✚ At 13:00 h, 5 PK sampling
- ✚ At 14:00 h, 6 PK sampling
- ✚ At 15:00 h, 7 PK sampling
- ✚ At 16:00 h, 8 PK sampling and Finger stick glucose and vital signs
- ✚ As from 16:00 h, lunch.
- ✚ At 18:00 h, 10 PK sampling
- ✚ At 20:00 h, 12 PK sampling and Finger stick glucose, Blood glucose determination and vital signs
- ✚ As from 21:00 h, dinner.

Day 2:

- ✚ At 00:00 h, 16 PK sampling
- ✚ At 8:00 h, 24.0-h PK sampling, Finger stick glucose, Blood glucose determination and vital signs.
- ✚ At 12:00 h, 28.0-h PK sampling.
- ✚ At 16:00 h, 32.0-h PK sampling, Blood glucose determination and vital signs.

Day 3:

- ✚ At 8:00 h, 48.0-h PK sampling, physical examination, vital signs, electrocardiogram and Safety laboratory
- ✚ Approximately at 10:00 h, temporary discharge from the clinical unit.

Day 4

- ✚ At 8:00 h, 72.0-h PK sampling.

Day 5

- ✚ At 8:00 h, 96.0-h PK sampling.



Day 6

✚ At 8:00 h, 120.0-h PK sampling.

Day 7

✚ At 8:00 h, 144.0-h PK sampling.

Day 8

✚ At 8:00 h, 168.0-h PK sampling.

✚ Approximately at 10:00 h, temporary discharge and free appointment.

9.2.2 Wash-out period

Wash-out period lasts for 14 days after administration in each period. 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.

9.2.3 Second period of treatment

Like the First period of treatment but after 14 days

9.2.4 Wash-out period

Wash-out period lasts for 14 days after administration in each period. 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.

9.2.5 Third period of treatment

Like the First period of treatment but after 28 days

9.2.6 Wash-out period

Wash-out period lasts for 14 days after administration in each period. 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.

9.2.7 Fourth period of treatment

Like the First period of treatment but after 42 days



9.2.8 End of Trial

- ✚ At 8:00 h, 168.0-h PK sampling.
- ✚ interview, physical examination (oral cavity examination, lungs, heart, abdomen and other organs, if necessary in case of symptoms),
- ✚ Vital signs measurement and follow-up of adverse events.
- ✚ Safety laboratory tests.
- ✚ Weight and height will be measured;
- ✚ Electrocardiogram.
- ✚ Blood samples will be taken for pregnancy tests for women of childbearing potential.

9.3 Baseline demographic and other characteristics

At screening, the following demographic data will be collected: date of birth, sex (gender), race, ethnicity

Specify any additional critical variables to be assessed such as:

- Information about previous and concomitant medications
- Medical history data
- Previous medication
- Weight, height and BMI
- Special diets
- For women, menstrual status, date of last menstrual period, and serum pregnancy test.
- 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

9.4 Safety Assessments

Clinical safety will be assessed by means of a complete physical examination (before the study and 48 and 168 h post-dose). On the other hand, throughout the study, subjects will be continuously monitored, including vital signs measurement (baseline and 4, 8, 12, 24, 32, 48 and 168 h post-dose) and an electrocardiogram will be performed (baseline, 48 and 168 h post-dose).

Safety by means of laboratory will be assessed by means of full laboratory tests (baseline and 48 h post-dose in every period). In blood analyses besides glucose (baseline, 4, 12, 24, and 32 post-dose), finger-stick glucose (Hematoglucotest) will be monitored (baseline, 2, 4, 8, 12 and 24 h post-dose).

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.

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 9.5.1.2). Reporting period for adverse events is described in section 8.4.1.3.

9.4.1 Adverse Event

9.4.1.1 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.

9.4.1.2 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:

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.

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.

9.4.1.3 Adverse Event's Severity

The following classification should be used.

Adverse events intensity or severity should be classified as follows:

- ✚ **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.

9.4.1.4 Laboratory Abnormal Findings and other Abnormal Findings throughout the Study

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, identified medical condition (for instance, anemia, and high ALT) should be reported as an adverse event instead of the abnormal value by itself.

9.4.1.5 Unexpected Adverse Event

An unexpected adverse event is any adverse event which specificity or severity are not foreseen 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 more specific or more severe than those described in the Investigator's Brochure should be considered "unexpected".

9.4.1.6 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.

9.4.1.7 Adverse Event of Special Interest

Hypoglycemia is a condition characterized by abnormally low blood glucose (blood sugar) 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 non-serious 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.

9.4.1.8 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.

Documentation should be supported by a record into 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, cause 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 details, 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.

9.4.1.9 Definition of Reporting Period for Adverse Events

Report of adverse events will start as per the informed consent form signature and until and continues through the trial tart as per the informed consent form signature and until end-of-trial examination (last contact with the subject).

9.4.1.10 Procedure for Reporting Adverse Events and Serious Adverse Events

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

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, 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.

Report of adverse events will be made according to the ICH GCP and the standard operating procedure, code: PPD [REDACTED] 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 and including all information collected, should be sent within 15 days. Report to the CNFV will be made by the Pharmacovigilance Officer at the PPD [REDACTED] [REDACTED]. 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 [REDACTED] [REDACTED], along with the clinical stage report to the CNFV.

PPD [REDACTED]
[REDACTED]
[REDACTED] [REDACTED]
[REDACTED]

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

PPD [REDACTED]

9.4.1.11 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 ICH GCP and the standard operating procedure, code: PPD [REDACTED] 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.

9.4.1.12 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.

9.4.2 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 as AE 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 9.4.1.10.

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 8.4.1.4. All abnormal outcomes should be reported (the observation period comprises 45 days following the delivery).

In case of pregnancy during the study, the subject should be withdrawn and no further IMP has to be given. 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 drug 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 Gliclazide, but insulin should be used to maintain blood glucose levels as close to normal as possible.

Pregnancy occurring during a patient's participation in a clinical trial, although not typically considered an SAE, must be notified to the sponsor within the same timelines as an SAE (within one working day) on a Pregnancy Monitoring Form. The outcome of a pregnancy should be followed up carefully and any abnormal outcome of the mother or the child should be reported. This also applies to pregnancies following the administration of the investigational product to the father prior to sexual intercourse.

Based on the outcome and the timing of the delivery, the pregnancies during the clinical trial can be categorized into the followings:

Female is study participant and becomes pregnant during study participation:

1. Normal outcome before end of study
2. Abnormal outcome before end of study
3. Normal outcome after end of study
4. Abnormal outcome after end of study



Female is partner of study participation and becomes pregnant during study:

5. Normal outcome before or after end of study
6. Abnormal outcome before or after end of study

In all of these situations, the Pregnancy Monitoring Form should always be filled out. However, only for situation #2, a SAE needs to be reported.

9.4.3 Clinical Laboratory Assessments

Blood samples for hematology:

1. Two (2) blood samples will be collected per period at times established in the schedule. Test tubes with EDTA K2, specifically designed for venipuncture, will be used.
2. Each sample will be 4 mL. Blood withdrawn to clean catheter should be disposed prior to the blood sample collection (purge).
3. A \pm 1 minute window is allowed for sampling.
4. Samples should be maintained at room temperature (between 20 and 25 °C [68 °F to 77 °C]).
5. Blood tubes will be labelled with Study No. (##), Case No. (C##), Sample No. (S##) and Visit No. (V#) according to the SOP, code PPD [REDACTED] Pre organization for conducting a study of interchangeability and biocomparability; PPD [REDACTED] Processing and packaging of biological samples for studies interchangeability and biocomparability. They will be kept at room temperature until their delivery to the clinical laboratory.

Samples for blood chemistry and glucose determination:

1. Two (2) blood samples for blood chemistry and 5 samples for glucose determination will be collected per period at times established in the schedule and in test tubes without anticoagulant specifically designed for venipuncture.
2. 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).
3. A \pm 1 minute window is allowed for sampling.
4. Samples should be maintained at room temperature (between 20 and 25 °C [68 °F to 77 °C]).
5. Blood tubes will be labelled with Study No. (##), Case No. (C##), Sample No. (S##) and Visit No. (V#) according to the SOP, code, PPD [REDACTED] Pre organization for conducting a study of interchangeability and biocomparability; PPD [REDACTED] Processing and packaging of biological samples for studies interchangeability and biocomparability. They will be kept at room temperature until their delivery to the clinical laboratory.

Blood samples for coagulation:

1. Two (2) blood samples will be collected per period at times established in the schedule in test tubes with sodium citrate specifically designed for venipuncture.
2. Each sample will be 3 mL. Blood withdrawn to clean catheter should be disposed prior to the blood sample collection (purge).
3. A \pm 1 minute window is allowed for sampling.
4. Samples should be maintained at room temperature (between 20 and 25 °C [68 °F to 77 °C]).
5. Blood tubes will be labelled with Study No. (##), Case No. (C##), Sample No. (S##) and Visit No. (V#) according to the SOP, code, PPD [REDACTED] Pre organization for conducting a study of interchangeability and biocomparability; PPD [REDACTED] Processing and packaging of biological samples for studies interchangeability and biocomparability They will be kept at room temperature until their delivery to the clinical laboratory.

Urine samples for urinalysis/ microscopic analysis / sediment:

1. Two (2) urine samples will be collected per period at the times established in the schedule.
2. Samples should be maintained at room temperature (between 20 and 25 °C [68 °F to 77 °C]).
3. Urine containers will be labelled with Study No. (##), Case No. (C##), Sample No. (S##) and Visit No. (V#) and will be kept at room temperature until their delivery 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 [REDACTED] 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.

▪ **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 [REDACTED] Tubes labeling and distribution in racks; BE-PNO-009 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.

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.

9.4.4 Vital Signs, Physical Examinations and other Assessments

At Screening and prior to each dosing, blood pressure, pulse, respiratory rate and 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.

In case it is necessary to be measured vital signs for some adverse event and coincide with the scheduled signs, the vital signs will be measured according to the current subject's condition until they are resolved, then the scheduled vital signs will be measured.

These values should be within the normal range (SBP between 80 and 129 mmHg and DBP between 50 and 89 mmHg, heart rate between 50 and 100 beats per minute) 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 as shown in the following:

Record of Vital Signs

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

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 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.

9.5 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: 0.00 (Pre-dose, 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 administration.



9.5.1 Body Fluids

Pharmacokinetics Blood Samples

- ✦ One blood sample 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 \pm 1 minute window is allowed for sampling during absorption and distribution phase, and up to \pm 3 minutes during the excretion phase.
- ✦ Each sample will be centrifuged at 2450 x g for 5 minutes at 4 °C [39.2 °F] (\pm 2 °C [35.6 °F]).
- ✦ Resulting plasma volume will be transferred into 1.5 or 2 mL cryotubes each.
- ✦ Plasma tubes will be perfectly identified with Study No. (##), Case No. (C##), Sample No. (S##) and Visit No. (V#). They will be kept deep frozen at a temperature lower than or equal to - 40 °C or lower, according to the standard operating procedures, code: PPD [REDACTED] Early organization for the conduct of an interchangeability and biocomparability study; and PPD [REDACTED] Processing and shipment of biological samples for interchangeability and biocomparability studies, PPD [REDACTED] Samples transportation from the clinical unit to the analytical unit; until their delivery at the analytical unit.

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.
- ✦ Samples Processing Responsible or 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 fridge. A reading will be made at least every 30 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.
- ✦ Samples should arrive deep frozen (- 20 °C [- 4.0 °F] as maximum) at the moment of the container bags check.
- ✦ A temperature log will be included in the trial master file.

Samples Receipt, Check and Storage by the Analytical Unit

- ✦ 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. 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 meanwhile the analytical unit and the clinical unit make an agreement about their disposition.

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 Labelling

Cryotubes will be identified with labels according to the standard operating procedure, code: **PP** , 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.

9.5.2 Pharmacokinetic calculations

Bioanalysis will be performed by analytical unit from the PPD [REDACTED].

PK parameters of Metformin and Gliclazide's plasma concentrations will be calculated and evaluated by the analytical unit from the PPD [REDACTED], according to their standard operating procedures and in accordance with the NOM-177-SSA1-2013.

Non-compartmental computation of PK parameters as well as descriptive statistical analysis for PK primary and secondary endpoints will be performed using Phoenix WinNonlin 6.3 software.

The primary PK variables will be calculated and listed for all subjects who provided sufficient concentration-time data.

9.6 Statistics

9.7 Sample Size

The sample size estimation is based on the CV intrasubject variability of both drugs. Because the pharmacokinetic and drug-drug interaction, evaluates changes in the bioavailability, the propose is the use the same determination for both aims.

The sample size is determined by the primary endpoint with the greatest variability under fasting conditions, AUC_{0-t} of Metformin (CV=23%). Assuming a mean treatment ratio of 0.95, and aiming at a power of 90% for the individual test, alpha=0.05 one-sided, 32 evaluable subjects are sufficient to demonstrate bioequivalence in the first step. The same arguments can be applied to the second step: Test absence of an effect of Gliclazide on the PK of Metformin, whereas the power for the third test is close to 100% with 32 subjects, due to the low variability of Gliclazide PK. Therefore, the overall power is still greater than 80% with 32 evaluable subjects. Considering a drop-out rate of approximately 20%, 40 subjects should be randomized.

According to NOM-177-SSA1-2013, this sample determination was realized with Chow & Wang (2001) equation

$$n \geq [t_{(\alpha, 2n-2)} + t_{(\beta, 2n-2)}]^2 \cdot \left(\frac{CV_{intra}}{\ln 0.8 - \ln \theta} \right)^2$$

Where:

$t(\alpha, 2n-2)$ and $t(\beta, 2n-2)$ considered the error α and β respectively

CV_{intra}: intra subject variability for crossover (or replicate crossover) study.

T_{theta}: 0.95: the target ratio in average BA between the two formulations expressed in percentage of the average reference

0.8: the least statistical power to detect (1-Power) differences between the Test and the Reference formulation (in this case the power expected is 90%)

9.8 Randomization

Forty male and female subjects will be included in this trial. Both sexes will be included with a minimum proportion of 30% for each sex at least 12 females and at least 12 males. Subjects will be randomly assigned to one of the 4 treatment sequences. Randomization of each subject to a treatment sequence will occur immediately before dosing on Day 1 of the first treatment period. The randomization list will be generated by the CRO based on the PPD [REDACTED] internal procedures and in compliance with the Mexican Official Standard NOM-177-SSA1-2013 using software in website www.randomization.com, in agreement with the sponsorures and in compliance with the Mexican Off drop-out before any drug administration, the new subject will be allocated the same randomization number. Subjects leaving the trial after they have received any of the drugs at least once might be replaced if the drop-out rate is higher than expected after discussion with the sponsor.

9.9 Outcome variables

9.9.1 Main outcome variable

To demonstrate Metformin/Gliclazide combination's bioequivalence at fixed dose as tablets (1.000 mg / 30 mg MR) compared to the concomitant administration of individual products (Metformin's tablet 1000 mg XR and Gliclazide tablets 30 mg MR), given as single dose to healthy volunteers in fasting state. The primary endpoints are AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} for Metformin and Gliclazide.

9.9.2 Secondary outcome variables

The secondary outcome variable are volume of distribution K_e , half-life elimination, clearance, and median residence time all these parameters will be presented only for informative reasons for both medications.

9.9.3 PK Analysis Set

The PK Analysis Set will include all subjects who have completed the trial with adequate trial medication compliance, without any relevant protocol violations with respect to factors likely to affect the comparability of PK results, and with sufficient evaluable data to determine primary endpoints (AUC_{0-t}, AUC_{0-∞} and C_{max}) for both analytes. If subjects received concomitant medication for the treatment of an AE, their inclusion in the PK population needs to be discussed on a case-by-case basis.

All PK analyses will be based on this Analysis Set.

9.9.4 Safety Analysis Set

All the deviations must be justified with statistical or scientific evidence and any change to the original statistical plan must be reported, in the master file of the study and in the statistical pharmacokinetic report and final report of the study

Subject data will not be replaced, any data missing will be considered as a non-existing data and likewise data cannot be eliminated from the statistical analysis, except in the following cases:

Research subjects with pre-dose plasma concentrations in the biological matrix.

If the pre-dose concentration is lower than 5% of the C_{max} value in a subject, the subject's data can be included without adjustment in all measurements and pharmacokinetic calculations. In the cases where the pre-dose value is greater than 5% of C_{max}, the research subject must be eliminated from all study evaluations.

Data elimination due to vomiting or diarrhea

Data from research subjects who experimented vomiting or diarrhea during the course of a clinical study for immediate release products can be eliminated from the statistical analysis if the vomiting or diarrhea occurred before 2 times the median of T_{max} or 2 times the value of T_{max} obtained in the research subject during a given period.

A research subject with very low plasma concentrations for study drugs

As established by the Mexican Official STANDARD NOM-177-SSA1-2013, also research subjects that in a crossover design do not provide assessable data, both from test drug and reference drug, should not be included in the statistical analysis.

A research subject is considered to have very low concentrations if the AUC is lower than 5% of the geometrical mean of the reference medication AUC (must be calculated without inclusion of data of the research subject with atypical values).

Data exclusion due to this reason will only be accepted under scientific justification and previous review of the case by the Federal Commission for the Protection against Sanitary Risks (Comisión Federal para la Protección contra Riesgos Sanitarios, COFEPRIS).

9.10 Statistical Analyses Description

9.10.1 General Considerations

Statistical analysis and analysis of PK parameters will be performed using Phoenix WinNonlin software Version 6.3 (PPD [REDACTED]).

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

Individual plasma concentration-time will be tabulated and plotted. Primary pharmacokinetics parameters, AUC and 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. All de calculations will be determinated in concordance with NOM-177-SSA1-2013

A complete description of the analyses will be given in the statistical analysis plan (SAP) into the protocol.

Statistical Analysis

9.10.2 Analysis of primary and secondary endpoints

The following sequential testing of a priori ordered hypotheses will be applied, as shown below. Bioequivalence test methodology will be used in all 3 steps:

1. Test bioequivalence of fixed combination and separate tablets
2. Test absence of an effect of Gliclazide on the PK of Metformin
3. Test absence of an effect of Metformin on the PK of Gliclazide

Bioequivalence in step1:

A mixed model will be applied to log-transformed C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ (both analytes) with treatment, period and sequence as fixed effects, and subject (sequence) as a random effect. Based on the residual error term 90% confidence intervals will be computed for the estimated differences Test – Reference, resulting in 90% confidence intervals for the Test/Reference ratios after back-transformation.

Drug-drug interaction in steps 2 and 3:

A mixed model will be applied to log-transformed C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ (one analyte) with treatment as fixed effect, and subject as a random effect. Based on the residual error term 90% confidence intervals will be computed for the estimated differences Test – Reference, resulting in 90% confidence intervals for the Test/Reference ratios after back-transformation.

1. Bioequivalence will be concluded, if all six 90% CIs for all six primary endpoints reside within the acceptance range [0.80 – 1.25]
2. Absence of an effect of Gliclazide on the PK of Metformin will be concluded, if all three 90% CIs for Metformin endpoints reside within the range [0.80 – 1.25]
3. Absence of an effect of Metformin on the PK of Gliclazide will be concluded, if all three 90% CIs for Gliclazide endpoints reside within the range [0.80 – 1.25]

Additionally, the primary endpoints will be descriptively analyzed and graphical displays (Box-whiskers-plots) will be prepared.

Statistical model in the variance analysis of mixed effects should be applied for log-transformed parameters C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ (one analyte) with TREATMENT as fixed effect and SUBJECT as randomized effect. Based on residual errors, confidence interval of 90 % will be built for estimated differences for test - reference, resulting in a confidence interval of 90 % for the test/reference ratio following transformation.

Using minimum mean squares for AUC and C_{max} , two-side t test will be performed (Schuirmann's test) by constructing confidence intervals of 90 % for test/reference ratios. If and only if intervals are within interval (80, 125), bioequivalence will be proven for both drugs.

Bioequivalence is concluded if 6 confidence intervals for the analytes are included into the bioequivalence limits.

8.11.4 Analysis of secondary endpoints

All other PK endpoints will be analyzed using descriptive methods. Graphical displays will be also prepared where appropriate.

9.10.3 Safety Analyses

All safety endpoints will be analyzed descriptively.

Vital signs measurements and ECG recordings will be individually listed by subject number, treatment and time point and flagged according to reference ranges. All variables will be presented by descriptive statistics by treatment and time point for the values obtained during the treatment periods and overall and by treatment sequence at screening and the end of study visit.

All hematology and biochemistry parameters, including hematoglucoest (HGT) results, will be listed and summarized using descriptive statistics on observed values by treatment and time point for the values obtained during the treatment periods and overall and by treatment sequence at screening and end of study visit. Urinalysis will be summarized in frequency tables.

In addition, shift tables for laboratory tests based on a classification of values as low, normal, or high with respect to the reference range could be summarized and presented by time point.

After coding of AEs according to the Medical Dictionary for Regulatory Activities (MedDRA) classification (current version) and assignment to a system organ class (SOC), all AEs recorded during the course of the trial will be listed by treatment and subject number and tabulated by MedDRA SOC and Preferred Term (PT).

The frequency of treatment-emergent AEs (TEAEs) will be summarized using the frequency of events and the number of subjects experiencing these events per treatment, PT, and SOC.

In addition, all TEAEs will be tabulated by intensity and relationship to drug per treatment.

An AE will be tabulated by intensity since the first investigational product administration of each period or if it occurred before administration and worsened during the period. The results will be presented as a frequency proportions or histograms.

9.10.4 Analysis of Further Endpoints

Individual concentrations of Metformin and Gliclazide in plasma will be listed and summarized by treatment. Individual plasma concentration-time profiles (linear and semi-logarithmic scales) will be plotted by treatment (showing all subjects simultaneously) and by subject (showing all treatments for a subject). Mean plasma concentrations will be plotted by treatment with SD using scheduled time points (linear and semi-logarithmic scales).

Demographic parameters (such as ethnic group, sex, age, height, weight, BMI) and other baseline characteristics (such as smoking history, diet, previous medications taken prior to screening visit, nicotine consumption, alcohol consumption, intake of caffeine or xanthine containing beverages) will be summarized by means of tabulated descriptive statistics for all subjects overall and by treatment sequence.

9.11 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 to increase reliability of the study conclusion. An analysis to identify outliers (extreme) values based on the student residual estimation among subjects will be performed according to lineal mixed model in Excel.

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 protocol, ethical principles established in the Declaration of Helsinki, ICH, GCP and established regulation NOM-177-SSA1-2012, 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 additional 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 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 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 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 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 [REDACTED] 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 an economical 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 will 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 protocol, case report form or informed consent form, a new approval should be got from both Committees.

10.6 Health Authorities

The clinical protocol 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 [REDACTED] 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. CRF's 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.
- Medical history.
- Previous concomitant therapies (including changes during the study).
- Study identification; i.e., subject's number.
- Study recruitment dates (informed consent) and visits to the site.
- Any medical exam and pre-defined clinical findings in this clinical protocol.
- All adverse events.
- Date when the subject withdraw his/her consent, including any reason for 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, 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 form the Sponsor.

11.4 Quality Management

Quality assurance is performed by means of follow-up to the standard operating procedures, codes: PPD [REDACTED] Elaboration of quality assurance report for clinical trials; PPD [REDACTED] First stage of quality control and quality assurance review during clinical studies conduct; PPD [REDACTED] Second stage of quality control and quality assurance review during clinical studies conduct; and PPD [REDACTED] 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 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 [REDACTED], Discrepancies identification and follow-up, 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

Protocol amendments can be issued by the Principal Investigator and agreed with the Sponsor or vice-versa. Substantial changes have to approve by the Ethics and Investigation Committee and by the Investigational Committee. Administrative changes (non substantial 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 study report according to the Mexican Official Standard which states tests and procedures to show that 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 KGaA, 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, but has to 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 outcome 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 research protocol which occur during study conduct should be documented. The Principal Investigator, the Study Sponsor have to be informed immediately. Ethics and Investigation Committed and to the Investigational Committee.

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 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.

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

Appendix 3: Drugs classification according to risks during pregnancy and breastfeeding (FDA)

A	Without apparent risks	Controlled studies in women do not show risk for the fetus during the first quarter; therefore, likelihood of fetal damage seems to be remote. <i>They can be used.</i>
B	Without apparent risks	Studies in animals do not show any risk for the fetus and there are no controlled studies in humans. Studies in animals do show adverse effects for the fetus; however, well controlled studies in pregnant women have not shown fetal risk. <i>Probably safe</i>
C	Undetectable risk	Studies in animals have shown that the drug has teratogenic or embryonic effects. There are no controlled studies or there are no studies available in animals or women. <i>Avoid them if there is any other option.</i>
D	Proven risk	There is positive evidence about fetal risk in humans but, in some cases (for instance, life-threatening situations or severe illnesses where more secure drugs cannot be used or which they turn to be not effective), benefits can make the drug acceptable in spite of the risks. <i>Avoid them if there is any other option.</i>
X	Contraindicated drugs	Studies in animals and humans have shown fetal abnormalities, or there is evidence of fetal risk based on the experience in human beings; or both situations apply and risk clearly overcomes possible benefit. <i>Contraindicated drugs</i>