PPD

PPD

"Prospective, single dose, randomized, open label, comparative, cross-study to establish bioequivalence between the new formulation and the approved formulation for Levothyroxine (Eutirox® from Merck, S. A. de C. V.) given as 3 tablets of 200 µg p.o. in healthy volunteers"

International non-proprietary name:	Levothyroxine
Reference drug:	Eutirox® from Merck, S. A. de C. V. (approved formulation)
Test drug:	Eutirox® from Merck, S. A. de C. V. (new formulation)
Project's general characteristics:	**Study code**: PPD / MS200125-0008; **Version**: 1.0; **Date**: 18th / october / 2018
Principal Investigator and protocol's author:	PPD
Study Monitor:	PPD
Sponsor:	Merck, S. A. de C. V.
Development phase:	Phase I (Bioavailability)
Clinical unit and accountable person:	PPD Ph. PPD
Analytical unit and accountable person:	PPD Ph. PPD

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Study investigators and other people involved

The undersigned acknowledge to conduct this study according to the provisions set forth in this protocol.

Clinical Unit

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Title:	Principal Investigator	Date:	
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Title:	Physician	Date:	
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Analytical Unit

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Sponsor

Name:	PPD	Signature:	
Title:	Accountable for the Study	Date:	

Committees

Ethics and Investigation Committee:

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Chairwoman
Ph. PPD Ext. PP
PPD filing number: PPD

Investigational Committee:

Dr. PPD
Chairman
Ph. PPD Ext. PP
PPD filing number: PPD

Clinical Laboratory

Clinical Laboratory and Sanitary Officer:

PPD
Ph. PPD

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Glossary and abbreviations

Glossary

Bioavailability Part of the drug absorbed in the blood stream following the dosing and time needed for this purpose (Mexican Official Standard [NOM, for its abbreviation in Spanish] NOM-177-SSA1-2013).

Bioequivalence Ratio between 2 drugs and equivalent dosage forms available to achieve similar bioavailability when given under the same conditions (NOM-177-SSA1-2013).

Pharmacokinetics Branch of pharmacology studying the process of a drug in the body. It is intended to elucidate the drug's effect from its dosing until it has been fully cleared from the body (Goodman & Gilman, 12th Edition).

Pharmacology Science studying the origin and physicochemical properties, dosage form, pharmacodynamics (biochemical and physiological effects, and mechanism of action), pharmacokinetics (absorption, distribution, biotransformation and excretion), as well as the therapeutic effect of the chemical substance interacting with living organisms. In the strict sense of the word, pharmacology is considered as the drugs study; and whether they are beneficial or toxic (Goodman & Gilman, 12th Edition).

Drug Any substance or mix of substances of natural or synthetic origin which have therapeutic, prophylactic or rehabilitation effect, and a dosage form identified as such by its pharmacological activity, and physicochemical and biological characteristics (NOM-073-SSA1-2005).

Abbreviations

A	Absorption
AE	Adverse Event
AUC	Area under the curve of serum concentration
AUC_{0-t}	Area under the curve of serum concentration from time 0 until the last sampling time
$AUC_{0-t(aj)}$	Area under the curve of serum concentration from time 0 until the last sampling time in pre dose corrected data
BC	Blood chemistry
BMI	Body Mass Index
°C	Celsius degrees
CBC	Complete blood count
CI	Confidence Interval
$C_{max\ (aj)}$	Maximum serum concentration in pre dose corrected data
C_{max}	Maximum serum concentration
CNFV	National Center of Pharmacovigilance
COFEPRIS	Federal Commission for the Protection against Sanitary Risks
CRF	Case Report Form
DBP	Diastolic Blood Pressure
dL	Deciliter
E	Elimination
EIC	Ethics and Investigation Committee
FDA	*Food and Drug Administration*
g	Gravity or grams (depending on the context)
h	Hour
HIV	Human immunodeficiency virus
IC	Investigation Committee
ICF	Informed Consent Form
IUPAC	*International Union of Pure and Applied Chemistry*
K_{el}	Elimination constant
kg	Kilogram

µg	Micrograms
m^2	Square meter
mg	Milligrams
min	Minute
mL	Milliliter
mmHg	Millimeters of mercury
PLM	Dictionary of Pharmaceutical Specialties
PPD	(SOP - Standard Operating Procedure)
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SC	Serum concentration
PPD	
TSH	Thyroid stimulating hormone
T3	Triiodothyronine
T4	Thyroxine
t	Time
t½	Elimination half-time
t_{max}	Time to reach the maximum serum concentration
UA	Urinalysis
VDRL	Venereal Disease Research Laboratory (serological screening test for syphilis)

1. Introduction

As per the pharmacokinetic characteristics, variable drugs' bioavailability, influence of the different techniques and pharmaceutical processes, including the possible differences in the quality and type of the formulation's ingredients, it is essential to perform comparative studies for new formulations. Therefore, it is important to assess the products under development by providing evidence about their appropriate and equivalent absorption and bioavailability with regards to the reference product; to ensure that the change of formulation will not be to the detriment of the subject's subsequent therapy, in addition to fulfill the legal requirements established by the health authorities.

1.1. Study Ethical Conduct

This protocol is compliant with the current regulation on clinical research, including the Good Clinical Practice (GCP), the ethical principles of medical research in human beings from the Declaration of Helsinki issued by the 64th General Assembly of the World Medical Association, Fortaleza, Brazil, October 2013, and the General Health Act and the Regulations of the General Health Act in the Field of Health Research, as well as with the International Ethical Guidelines for Health-related Research Involving Humans, Prepared by the Council for International Organizations of Medical Sciences (CIOMS) in collaboration with the World Health Organization (WHO), Geneva 2016.

According to the provisions of the Regulations of the General Health Act in the Field of Health Research, second title, chapter I, section 17, part III issued in the Official Gazette of April 2nd, 2014, this study is considered as a study with risk higher than minimum.

1.2. Levothyroxine Pharmacology

1.2.1. Levothyroxine's chemical structure

Levothyroxine systematic name is (*PubChem CID 23666112- Levothyroxine Sodium)*:

(2S)- 2-amine-3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl] sodium propanoate

Levothyroxine's molecular formula is: $C_{15}H_{10}I_4NNaO_4$. Its molecular weight is 798.856 g/mol and its structural formula is (*PubChem CID 23666112- Levothyroxine Sodium)*:

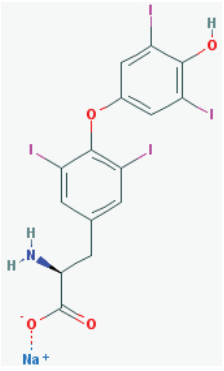


Figure 1.- Levothyroxine

1.2.2. Levothyroxine pharmacokinetic parameters

TABLE No. 1

No.	DOSES	C_{max}	t_{max} (h)	t½ (h)	$AUC_{0-\infty}$	Reference
1)	600 µg*	11.58 (2.10) µg/dL	2.50 (4.50)	403.84 (16850.26)	14447.11 (36474.98) µg·h/dL	*Abib et al., (2010)*
2)	600 µg**	11.53 (2.40) µg/dL	2.00 (23.02)	348.30 (6309.87)	10679.17 (17326.22) µg·h/dL	*Abib et al., (2010)*
3)	600 µg*	112.0 ± 17.3 ng/ml	3.9 ± 1.6	ND	◊ 4359.3 ± 619.3 ng h/ml	*Koytchev & Lauschner, (2004)*
4)	600 µg**	113.4 ± 18.5 ng/ml	4.6 ± 2.0	ND	◊ 4463.8 ± 694.7 ng h/ml	*Koytchev & Lauschner, (2004)*

1) and 2) Data reported as mean (standard deviation); 3) and 4) Data reported as mean ± standard deviation; * Test drug; ** Reference drug; ◊ AUC_{0-48h}; NA, not available.

1.2.2.1. Absorption

Levothyroxine is synthetically produced, but it is identical to the T4 secreted by the thyroid. Levothyroxine's gastrointestinal absorption is approximately 80 % and does not change in hypothyroid or euthyroid state. Absorption takes place throughout the human small bowel but decreases distally. Following levothyroxine's intake, T4 serum levels reach its peak within 2 to 4 hours and remain above the baseline level for 6 h. Increase of T3 serum levels following levothyroxine's administration is slow due to the time required for the T4 conversion into T3 (*Koytchev & Lauschner, 2004*).

1.2.2.2. Distribution

Levothyroxine has a limited volume of distribution of 11.6 L in healthy volunteers. Both T4 and T3 bind in a higher ratio of 99.8 % of plasma protein, mainly the thyroxine binding globulin (TBG), and in a lesser degree to the thyroxine binding pre-albumin and to the albumin (*Guidance to Establish the Interchangeability of Oral Solid Drugs Containing Levothyroxine Sodium, COFEPRIS, 2017).*

1.2.2.3. Metabolism

T4 metabolism is carried out by means of de-iodination sequential reactions. Approximately 80 % of the T4 daily dose is transformed into T3 which is the active hormone and into reverse T3 (rT3), in an approximate ratio of 50 % for each of them. In turn, T3 hormone is metabolized into diiodothyrosine (T2) and iodotironamine (T1) (*Guidance to Establish the Interchangeability of Oral Solid Drugs Containing Levothyroxine Sodium, COFEPRIS, 2017).*

1.2.2.4. Excretion

Levothyroxine's elimination is produced by conjugation with glucuronic and sulfuric acid, and excretion in the bile. Thyroid hormones release by means of bacterial hydrolysis in the bowel and partial re-absorption lead to enterohepatic circulation. Approximately 20 - 40 % of thyroxine is cleared in the feces (*Koytchev & Lauschner, 2004*).

T4 elimination half-life is approximately 6.2 days in healthy volunteers, meanwhile T3 half-life is of approximately 1 day (*Guidance to Establish the Interchangeability of Oral Solid Drugs Containing Levothyroxine Sodium, COFEPRIS, 2017).*

1.2.3. Pharmacodynamics (mechanism of action)

Levothyroxine Sodium is a levothyroxine sodium salt, a synthetic levoisomer of thyroxine (T4), similar to the endogenous hormone produced by the thyroid gland. Thyroxine is de-iodinated by 5' de-iodinase to form triiodothyronine (T3) in the peripheral tissues. T3 enters the cell and binds to nuclear thyroid hormone receptors, and the hormone-receptor complex in turn triggers gene expression and produces proteins required in the regulation of cellular respiration, thermogenesis, cellular growth and differentiation, and metabolism of proteins, carbohydrates and lipids. T3 also possesses cardiac stimulatory effect. (*PubChem CID 23666112-Levothyroxine Sodium*).

1.2.4. Pharmacological properties

Hypothyroidism: Levothyroxine is indicated in replacement therapy in congenital or acquired hypothyroidism, of any etiology. Specific indications include: Primary hypothyroidism (thyroid), secondary (pituitary) or tertiary (hypothalamic) and subclinical hypothyroidism. Pituitary suppression of TSH: in the treatment or prevention of various types of euthyroid goiters, including thyroid nodules, chronic or subacute lymphocytic thyroiditis (Hashimoto's thyroiditis), multinodular goiter and as adjuvant to radioiodine or surgical therapy for cancer management well-differentiated thyroid-dependent thyrotropin (*PLM, 2016 - Eutirox®*).

1.2.5. Adverse reactions

Side effects described for levothyroxine therapy based on its frequency are the following (*Drugs & Medications – Levothyroxine Sodium*):

- **Common:** in women difficulty to get pregnant.
- **Uncommon:** poor calcification and bone density, hypertension and weight loss.
- **Rare:** abnormal heart rhythm, abnormal liver function tests, angina, cardiac arrest, tachycardia, heart failure or heart attack, hives, allergic dermatitis, mood swings, pseudotumor cerebri, seizures, rash, lack of breath, pulse changes, chronic sleep difficulty, diarrhea, irritability, excessive sweating, anxiety, fever, hair loss, headache, palpitations, appetite increase, involuntary tremors, irregular menstruation, low energy, muscular weakness, nervousness, abdominal cramps, temporary redness of the face and neck, vomiting.

The CIOMS classification (Council for International Organizations of Medical Sciences in collaboration with the World Health Organization [WHO]) for drug's adverse reactions based on their frequency are the following:

- Very frequent or very common: they occur with a frequency ≥ 1 case for each 10 patients who receive the drug. It is expressed as ≥ 1/10.

- Frequent or common: they occur with a frequency ≥ 1 case for each 100 patients who receive the drug, but less than 1/10. It is expressed as ≥ 1/100 and < 1/10.
- Infrequent or uncommon: they occur with a frequency ≥ 1 case for each 1,000 patients who receive the drug, but less than 1/100. It is expressed as ≥ 1/1,000 and < 1/100.
- Rare: they occur with a frequency ≥ 1 case for each 10,000 patients who receive the drug, but less than 1/1,000. It is expressed as ≥ 1/10,000 and < 1/1,000.
- Very rare: they occur with a frequency less than 1 case for each 10,000 patients who receive the drug. It is expressed ad < 1/10,000.

1.2.6. Precautions with regards to carcinogenicity, mutagenicity, teratogenicity and fertility

Thyroid hormones do not cross the placental barrier. Based on clinical experience, levothyroxine does not cause fetal abnormalities when given during pregnancy. No conclusive studies to determine mutagenicity and carcinogenicity have been conducted; and despite the apparent relationship between prolonged thyroid hormones therapy and breast cancer, a true validation for such statement does not exist (*PLM, 2016 - Eutirox®*).

Levothyroxine belongs to the FDA's category A for the risk classification during pregnancy and breastfeeding (*Drugs.com - Levothyroxine Pregnancy and Breastfeeding Warnings*) (See Appendix 12.4).

1.3. Problem statement

Study rationale

Congenital hypothyroidism is the most frequent cause of preventable intellectual disability. Incidence in Mexico is of 1:2,729 newborns. More than 90 % of congenital hypothyroidism are of primary origin and its treatment consists of thyroid hormone deficiency replacement by adjusting the dose according to patient's age and clinical and biochemical conditions (*Rojas et al., 2009*).

Currently, the optimal therapy for hypothyroidism requires the thyroid hormone deficiency replacement with synthetic levothyroxine. The drug is used as interventional therapy to suppress the secretion of the thyroid stimulating hormone (TSH) in patients with nodular thyroid disease or thyroid cancer. As the evident hypothyroidism incidence occurs in 1.5 % to 2 % of women and in 0.2 % of men, and its incidence increases with age to 6 % and 2.5 % in women and men older than 60 years old respectively, levothyroxine is one of the most prescribed drugs in the United States (*Koytchev & Lauschner, 2004*).

Under the concept that the biological effect of a drug depends on the concentration reached in the effector organ, and based on its steadiness in the blood concentration, it is accepted that the pharmacokinetic equivalence between two drugs is evidence that the same efficacy and clinical safety will be achieved. Therefore, bioavailability studies should be subject to a clinical research protocol where confounding variables affecting the study basic characteristics such as oral dosing formulation: rate and extent of absorption are controlled by an experimental design. This absorption process characteristics are measured by the pharmacokinetic parameters of t_{max} and C_{max} for the rate and the AUC_{0-t} for the extent.

As the goal is to prove the lack of significant differences in the pharmacokinetic parameters where each subject is his/her own control, the chosen experimental design is one of those suggested by the NOM-177-SSA1-2013, crossed, open label, two sequences and two periods of treatment (scheme A - B and B - A), and single dose. Section 8.4.8.1 of this Standard states that drugs with a long half-life, crossed or parallel designs should include a limited sampling for at least 72 hours. However, prior studies showed that sampling times longer than 48 hours do not allow to characterize differences between exogenous and endogenous levothyroxine concentrations with certainty. Therefore, bioequivalence studies for this drug should be truncated up to 48 hours (*Guidance to Establish the Interchangeability of Oral Solid Drugs Containing Levothyroxine Sodium, COFEPRIS, 2017).* According to the, a 48-h sampling time, which is compliant with the Mexican health authorities' requirement, is proposed. In addition, it allows to characterize at least the 80 % of the AUC, by having 26 total samples and a wash-out period of 35 days between the two study periods.

Besides the inclusion and exclusion criteria, confounding variables to be controlled are: fasting state (at least 10 hours before the drug intake); morning time for the administration; volume and type of liquid for the drug intake; timing, type and quantity of food; liquid intake prior to the breakfast; restricted physical activity for a minimum that does not alter the heart rate; orthostatic or seated position till reaching the stated time for t_{max}; abstinence of caffeine or theophylline containing products; controlled access to restrooms until the t_{max} is reached. Additionally, concomitant drugs are not allowed, except for the medical prescription for to adverse events. The occasional use of acetaminophen (maximum of 1000 mg per day, for three days) is allowed. While complying with the permitted dose, the use of paracetamol will not be reason for exclusion of the subject, nor for the study or pharmacokinetic analysis.

Literature information states that the systemic levels of levothyroxine (T4) show a better correlation with the T4 levels in the triiodothyroxine (TE) or thyroid stimulating hormone (TSH) site of action. Therefore, it is the most appropriate to establish the drug's efficacy and safety. Despite the TSH sensitivity to changes in the level of thyroid hormone, the TSH is not appropriate to establish the bioequivalence between formulations since there is a significant delay between the exogenous levothyroxine dosing and the observed changes in the TSH levels (*Guidance to Establish the Interchangeability of Oral Solid Drugs Containing Levothyroxine Sodium, COFEPRIS, 2017).*

Since levothyroxine is and endogenous compound, a high relation of exogenous levothyroxine concentrations with regards to the baseline concentrations of endogenous levothyroxine should be guaranteed to distinguish the real differences between the formulations. Therefore, over-therapeutic doses of levothyroxine are encouraged, as well as the pre-dosing study drug sampling to determine the baseline values of the endogenous levothyroxine (*Guidance to Establish the Interchangeability of Oral Solid Drugs Containing Levothyroxine Sodium, COFEPRIS, 2017).* According to the aforementioned, the dose to be used in the study is the recommended one, which is equivalent to 600 µg of levothyroxine. Three pre-dose drug samples will be taken to determine the baseline levels of endogenous levothyroxine. Levothyroxine (T4) serum concentrations measurement will be carried out. C_{max} and AUC_{0-t} parameters will be used for the statistical analysis of bioequivalence, by means of 90 % confidence intervals construction for geometric means of these pharmacokinetic parameters. The statistical analysis will be made using the corrected values only. In both pharmacokinetic parameters, equivalence margins will be of 80 % to 125 % according to the *Guidance to Establish the Interchangeability of Oral Solid Drugs*

Containing Levothyroxine Sodium, (COFEPRIS, 2017). There are no data on variation of serum levels of levothyroxine due to gender; therefore, this study will be carried out in male and female healthy volunteers.

2. Primary Objective

To establish the bioequivalence between the new and approved levothyroxine's formulation in tablets of 200 µg (Eutirox® from Merck, S. A. de C. V.) in healthy volunteers for the marketing approval of the new formulation in all its strength. The lower strengths will be justified with the biowaiver criteria described in NOM-177-SSA1-2013.

2.1. Secondary objectives

To assess the pharmacokinetics parameters of levothyroxine at a total dose of 600 µg (3 x 200 µg) of the test and reference drugs.

To assess the safety and tolerability of levothyroxine at a dose of 600 µg (3 x 200 µg) of the test and reference drugs.

The study objectives and endpoints are described in Table 2.

TABLE No. 2 Study Objectives and endpoints

Primary Objective	Endpoints
• To establish the bioequivalence between the new and approved levothyroxine's formulation in tablets of 200 µg (Eutirox® from Merck, S. A. de C. V.) in healthy volunteers for the marketing approval of the new formulation in all its strength. The lower strengths will be justified with the biowaiver criteria described in NOM-177-SSA1-2013	• Primary endpoints are baseline-adjusted $C_{max(aj)}$ and $AUC_{0-t\ (aj)}$of levothyroxine (T4) in serum after dosing of 600 µg levothyroxine with either the test product or the reference product
Secondary objectives	**Endpoints**
• To assess pharmacokinetic parameters of levothyroxine • To assess the safety and tolerability of levothyroxine 200µg for both test and reference products.	• Levothyroxine (T4): t_{max}, t1/2, AUC_{0-t} and C_{max}. • Safety assessments: • Physical examination • Vital signs (body temperature, blood pressure, Breathing frequency and pulse rate) • Complete blood count; 4-panel chemistry; urinalysis; liver function tests; HIV, hepatitis B and C, and VDRL test; and qualitative urine test for illicit drugs, and pregnancy test for women. Thyroid profile. • Electrocardiogram (ECG) • Recording of Adverse Events (AEs)

3. Statistical hypothesis

H_0: Drug A and drug B are not bioequivalent.

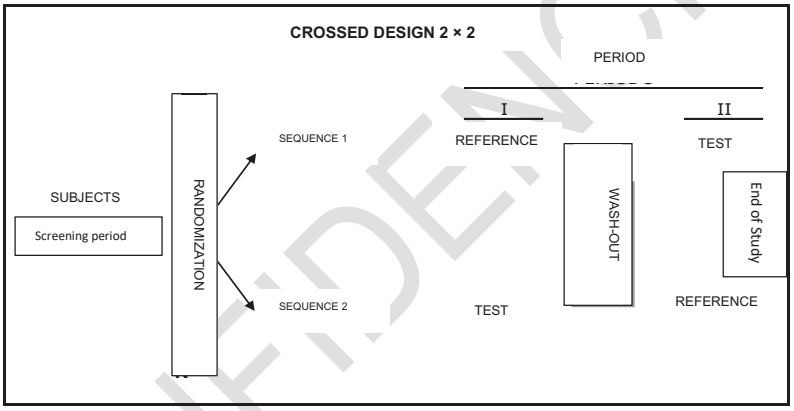
H_1: Drug A and drug B are bioequivalent.

4. Investigational plan

4.1. Experimental design and study plan

Single dose design will be used with 44 subjects in fasting state, two periods, two sequences, open-label, crossed in scheme blocks A-B and B-A, randomized, longitudinal, comparative and prospective, with a 35-day wash-out period between the two study periods. Treatment groups will be balanced, with the same number of subjects who will be randomly assigned to the study drug sequences of administration.

All data from participant subjects should be analyzed and included in the statistical analysis, if the fulfill with the established criteria.



NOM-177-SSA1-2013

Selection period (Screening)	Clinical phase			
	Period 1	**Washout**	**Period 2**	**Clinical monitoring End of Study**
30 to 2 days before the start of Period 1	From approx.12 hours pre-dose until 48 h sampling post-dose	At least 35 days	From approx. 12 hours pre-dose until 48 h sampling post-dose	At least 14 days after the dose in period 2

Subjects will be randomized to one of the 2 sequences by a randomization plan completed before first dosing

4.2. Study Population Screening (30 to 2 days before start of period 1)

- Invitation-made recruitment by open call.
- Inclusion of 44 subjects in the study according to the protocol's criteria.

4.2.1. Inclusion Criteria

All the following criteria should be met to be included in the study:

- Subjects who have provided their written consent prior to any study-related activity.
- Male and female (non-pregnant women) healthy volunteers, Mexican (NOM-177-SSA1-2013).
- From 18 to 55 years old (NOM-177-SSA1-2013).
- Body mass index from 18 to 27 kg/m^2 (NOM-177-SSA1-2013).
- Normal vital signs (the measurement will be made according to the instructive PPD):
  - ✓ 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 80 mmHg
  - ✓ Temperature between 36.0 °C and 37.0 °C.
- Normal electrocardiogram [ECG]. No abnormalities are allowed, even though they are not relevant (PR, QRS, QT, QTcF should be within normal range; no conduction abnormalities are allowed, etc.)
- All values in blood and urine tests should be within the normal range or showing no clinically relevant deviation as judged by the Investigator, in the following:
  - ✓ Complete blood count (CBC): hematocrit and hemoglobin mandatorily above the lower level.
  - ✓ Liver function tests, including: total bilirubin, direct bilirubin, indirect bilirubin, glutamic oxaloacetic transaminase, pyruvic glutamic transaminase, total protein, albumin, globulin and albumin-globulin ratio.
  - ✓ Chemistry (C) including at least: glucose, urea, creatinine and uric acid.
  - ✓ Lipid panel, including total cholesterol and triglycerides.
  - ✓ Urinalysis (UA).
  - ✓ Subjects with isolated data, reported as out of range and not related each other, can be included in the study after documenting that signs and symptoms are not related each other at the screening and who do not have personal history of any related disease.
- Subjects with thyroid panel results within normal range (T3 and T4 total and free, as well as TSH should be within the normal range).
- Non-smoker at least in the last 3 months.

- All child-bearing potential women who are not pregnant or who are breastfeeding, and who are using any highly effective contraceptive method (defined as those which alone or combined, which failure rate is low; i.e., less than 1 % a year if used continuously and correctly) for at least the first dose and after the last one, can take part in in the study. Barrier methods and intrauterine device are considered as standard contraceptive methods. Women using hormonal methods will not be included. Post-menopausal women can be included (i.e., those amenorrhoeic for at least 12 consecutive months following their last cycle) or those who are surgically sterile or who have undergone hysterectomy at least 6 months prior to their participation in their study.

4.2.2. Exclusion Criteria

Subjects will not be eligible for the study if meet one or more of the following exclusion criteria:

- Participation in the clinical study within 90 days prior to the first dose of the study drug.
- Subjects with history of hypersensitivity or allergy to the study drug or its excipients; history or current asthma or any severe allergy (which requires hospitalization or prolonged therapy), allergy or intolerance to any food that, in the opinion of the investigator, poses a safety risk (allergy to iodine, etc.)
- Subjects with history of cardiovascular, renal, liver, metabolic, gastrointestinal, neurological, endocrine, hematopoietic (any type of anemia) conditions, mental disorder or organic abnormalities that may affect the pharmacokinetic study of the study drug.
- Any medical or surgical condition, including findings in the medical history or clinical assessment prior to the study, that in the opinion of the investigator poses a risk or contraindication for the subject's participation in the study and may affect the study objectives, conduct or analysis.
- History or presence of alcohol abuse (average daily intake not higher than 3 units or weekly intake not higher than 21 units; 1 unit is equivalent to 340 mL of beer, 115 mL of wine or 43 mL of prepared drinks), psychoactive substances or chronic use of drugs.
- Subjects who have been exposed to agents knows by inducing or inhibiting the liver enzymatic systems or who have taken potentially toxic drugs within the last 30 days to the study start-up.
- Subjects who take drugs affecting the metabolism of the thyroid hormone, such as: oral contraceptives, hormonal implants, parenteral hormones, steroids, anabolic drugs, androgens, etc., or any drug affecting the levothyroxine's bioavailability such as the proton pump inhibitors or multivitamins, nutritional supplements or herbal products that may affect the study, except for the occasional use of paracetamol.
- Subjects who have been hospitalized for any reason within the 60 days prior to the study start-up or who have been severely ill within the last 30 days prior to the study start-up.
- Subjects who have donated or lost 450 mL of blood within the last 60 days prior to the study start-up.
- Subjects non- smokers, who have smoked tobacco, cigarettes or consumed coffee, snuff or drinks containing xanthines such as caffeine, (tea, cocoa, chocolate, matte, cola, etc.) theobromine, theophylline, among others, affecting the pharmacokinetics of the drug in assessment, drinking alcohol, or charcoal-grilled foods consumed within twenty-four hours prior to administration the dose of medication.

- Screening biosafety positive tests for the human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) and syphilis (VDRL).
- Positive result in the abuse drugs screening tests, such as: amphetamines, benzodiazepines, cocaine, methamphetamines, morphine and tetrahydrocannabinoids.
- Presence of alcohol in breath test.
- Women pregnancy postivie tests (qualitative and quantitative) at the screening and inclusion in each period.
- Consumption of grapefruit, oranges, cranberries, or their juices within 48 hours prior to the study drug dosing, until the last sampling in each period.
- Breastfeeding women.
- Abnormal findings in the medical history that, as per the investigator's judgment, poses a risk or contraindication for the study subject's participation; or that might interfere with the study objectives, conduct or analysis.
- Any case where the Principal Investigator thinks that the lack of compliance to the protocol may the study results questionable (serious adverse reaction or serious adverse event, subject's indiscipline, lack to compliance to the diet, emesis within the established time for the t_{max}).
- Difficulties to follow the protocol's requirements, instructions or study-related restrictions; e.g., uncooperative behavior, impossibility to return for the visits and likelihood of not completing the study.
- Impossibility to communicate or cooperate with the Investigator (language issues, illiterate subjects, poor mental capacity) or to follow the study requirements, including diet restrictions.
- If the subject is the Principal Investigator or any Sub-Investigator, assistant, coordinator, or relative to any of the team members directly involved in the study conduct.

Additionally, participant subjects in the study are free to abandon the study at any time and they will only be requested to inform the reason of their withdrawal.

4.3. Study Early Termination

- This study can be temporally or definitively terminated based on the Sponsor, Principal Investigator or Ethics and Investigation Committee or Investigation Committee's decision, according the Ministry of Health guidelines.
- Causes may be different, among others the frequency and type of adverse events which make a considerable number of subjects to take the decision of terminating the study; new drug's information which states that the subjects' safety or study conduct might be affected; or by reasoned decision by the Ethics and Investigation Committee, Investigation Committee and the Ministry of Health.
- In the event of temporary or early termination, study subjects will be immediately informed and an appointment will be scheduled to inform them about the final decision about the study course. For definitive termination, the Principal Investigator will inform this decision to the study subjects and will perform the termination procedures.

- If the decision is taken by the Sponsor or the Principal Investigator, they should document the decision and inform in writing, with a detailed explanation about the temporary or definitive termination, to the Ethics and Investigation Committee, to the Investigation Committee and to the Ministry of Health.
- Study drugs will be kept safely and will be disposed (return or storage) as agreed with the Sponsor.

4.4. Study Procedures

4.4.1. Subjects Screening

Once the informed consent form is signed, subjects' eligibility will be checked according to the inclusion and exclusion criteria. Confirmation of the medical chart and its update will be made according to the standard operating procedure, code: PPD, at the PPD If the subject already has a medical chart, the medical history will only be updated.

Subjects should have:

- Laboratory tests within 30 days prior to the study start-up.
- Electrocardiogram within 30 days prior to the study start-up.

4.4.2. Study Periods

To confirm that the subject is still a suitable candidate for the study at the entrance of each period, a clinical assessment consisting of interrogation by apparatus and systems with a physical exam and an electrocardiogram will be carried out. Urine samples will be taken for abuse drugs screening; for women, a blood sample will be taken for the pregnancy tests. In addition, a breath alcohol test will be done.

At the end of each period, a clinical assessment consisting of an interrogation, physical exam, vital signs measurement and adverse events follow-up will be done.

Subjects should inform to the medical staff in charge of the study conduct about any symptom they have. Likewise, the medical staff will enquire in each period to the subjects about symptoms since the first period and following the dosing. Should they report any, the medical staff should provide treatment to the subject and notes to the appropriate documents will be made.

Prior to the dosing, blood pressure (seated position), pulse, respiratory frequency and temperature will be measured to each subject. These values should be within the normal ranges (SBP between 80 and 129 mmHg and DBP between 50 and 80 mmHg) in order the subjects keep on participating in the study. Same measurements will be taken in every shift following the dosing in each phase. Vital signs will be taken as shown in Table No. 3.

TABLE No. 3

Vital Signs Record

First Period	Second Period
Admission	Admission
Pre-dose	Pre-dose
Morning shift (2 to 3 h after administration)	Morning shift (2 to 3 h after administration)
Evening shift (6 to 8 h after administration)	Evening shift (6 to 8 h after administration)
Night shift (11 to 13 h after administration)	Night shift (11 to 13 h after administration)
Discharge (23 to 24 h after administration)	Discharge (23 to 24 h after administration)
Ambulatory sample (36 and 48 hours)	Ambulatory sample (36 and 48 hours)

If any subject has clinically significant changes, out of the normal ranges, in his/her blood pressure and pulse during post-dose confinement, he/she will be closely checked by the medical staff and appropriate supporting measures will be taken according to the his/her condition until resolution. Should these changes persist, his/her participation in the study will be checked by the Principal Investigator.

Due to operations issues, the Principal Investigator may change the study start-up (from the study drug intake) in 1 hour, if it does not affect the study result.

4.4.2.1. Drugs to be given

Drugs to be given to the subjects in each of the study visits will be given by the study Sponsor.

- International non-proprietary name: Levothyroxine Sodium.
- Reference drug: Eutirox® (approved formulation).
  - Dosage form: tablets.
  - Qualitative-quantitative formula: each tablet contains levothyroxine sodium 200 µg.
  - Expiry date: The expiry date must not be before the end of the study.
  - Sanitary approval number: 297M89, SSA IV.
  - Manufacturer: Merck, S. A. de C. V.
  - Distributor: Merck, S.A. de C.V.
  - Marketing Authorization Holder: Merck, S.A. de C.V.
- Test drug: Eutirox® (new formulation).
  - Dosage form: tablets.
  - Qualitative-quantitative formula: each tablet contains levothyroxine sodium 200 µg.
  - Expiry date: The expiry date must not be before the end of the study.

- Manufacturer: Merck, S. A. de C. V.
- Distributor: Merck, S. A. de C. V.

- Dose to be given: 600 µg (3 tablets of 200 µg), orally.

4.4.2.2. First Treatment Period

- Admission in the afternoon of the day before dosing (approximately at 3pm).
- Dinner at least 10 hours before the dosing.
- Catheter insertion (as chosen).

First Day:

- At 06:00 h, grooming and intake of 250 mL of water.
- Between 06:30 and 07:30 h, catheter insertion.
- As per 07:30 h, first pre-dose sample (- 0.50 h); as per 07:45 h, second pre-dose sample (-0.25 h); and as per 07:55 h, third pre-dose sample (0 h).
- As per 08:00 h, study drug or reference drug dosing with 250 mL of room temperature water, according to the standard operating procedure, code: PPD
- From 08:30 to 20:00 h, sampling according to the schedule.
- As per 12:00 h, breakfast.
- As per 16:00 h, lunch.
- As per 21:00 h, dinner.

Second Day:

- At 08:00 horas, 24.0 h sampling.
- Approximately at 10:00 h, discharge from the clinic.
- At 20:00 h, 36.0 h ambulatory sampling.

Third Day:

- At 08:00 h, 48.0 h ambulatory sampling.
- Approximately at 10:00 h, temporary discharge with open appointment.

4.4.2.3. Wash-out Period

Wash-out period length is of 35 days starting at the drug's dosing in the previous period. It is intended to remove the previous dose before giving the next one and fulfills the criterion of the NOM-177-SSA1-2013 of at least 7 half-lives for the study drug.

4.4.2.4. Second Treatment Period

- Admission the afternoon of the day before dosing (approximately at 3pm).
- Dinner at least 10 h before the dosing.
- Catheter insertion (as chosen).

First day:

- At 06:00 h, grooming and intake of 250 mL of water.
- Between 06:30 and 07:30 h, catheter insertion.
- As per 07:30 h, first pre-dose sample (- 0.50 h); as per 07:45 h, second pre-dose sample (-0.25 h); and as per 07:55 h, third pre-dose sample (0 h).
- As per 08:00 h, study drug or reference drug dosing with 250 mL of room temperature water, according to the standard operating procedure, code: PPD
- From 08:30 to 20:00 h, sampling according to the schedule.
- As per 12:00 h, breakfast.
- As per 16:00 h, lunch.
- As per 21:00 h, dinner.

Second Day:

- At 08:00 horas, 24.0 h sampling.
- Approximately at 10:00 h, discharge from the clinic.
- At 20:00 h, 36.0 h ambulatory sampling.

Third Day:

- At 08:00 h, 48.0 h ambulatory sampling.
- Approximately at 10:00 h, temporary discharge with open appointment.

4.4.2.5. Clinical Follow-up

All study subjects will be followed-up in an outpatient basis, as per the last drug's dosing, with open appointment or telephone contact as per the Investigator's opinion, for 14 days after last administration.

4.4.2.6. Final visit

14 days after the last administration, the subjects will be cited for their final visit in which the following evaluations will be made:

- Sampling for clinical laboratory: equal to the tests carried out in the screening.

- Control electrocardiogram.

- Medical review: will include directed interrogation (including AEs) and physical examination.

4.4.2. 7. End of the study

Any clinically relevant deviation from the initial findings for all subjects should be monitored until they have returned to normal, are no longer considered clinically relevant or can be explained.

Adverse events that were presented during the follow-up period and continued unresolved in the final visit, should be followed until resolved, are no longer considered clinically relevant or can be explained.

The study will end when the last subject has been subjected to the activities described in the final visit and the laboratory or cabinet findings or adverse events, if detected, have been explained and closed by the investigator.

4.4.3. Blood sampling schedule

Clinical Stage

NUMBER OF SUBJECTS:	44	SEX:	Male or female
BIOLOGICAL MATRIX:	Serum	DOSAGE FORM:	Tablets
PHARMACEUTICAL FORM:	Levothyroxine sodium 200 µg	DOSES AND NO. OF UNITS:	200 µg (3 tablets)
WASH-OUT PERIOD:	35 days	ANTICOAGULANT:	Does not apply
BLOOD VOLUME EXTRACTED PER SAMPLE:	10 mL	TOTAL OF SAMPLES PER SUBJECT IN EACH PERIOD:	26

Sampling time interval

Stage	Sample	Time	Unit
Pre-dose	01	- 0.50	h
Pre-dose	02	- 0.25	h
Pre-dose	03	0.00	h
A	04	0.50	h
A	05	1.00	h
A	06	1.50	h
A	07	2.00	h
A	08	2.50	h
~ C_{max}	09	3.00	h
~ C_{max}	10	3.25	h
~ C_{max}	11	3.50	h
~ C_{max}	12	3.75	h
~ C_{max}	13	4.00	h
~ C_{max}	14	4.25	h
~ C_{max}	15	4.50	h
~ C_{max}	16	4.75	h
~ C_{max}	17	5.00	h
E	18	5.50	h
E	19	6.00	h
E	20	6.50	h
E	21	8.00	h
E	22	10.00	h
E	23	12.00	h
E	24	24.00	h
E	25	36.00	h
E	26	48.00	h

Total number of samples			
No. of cases	Samples	Periods	Total
44	26	2	2,288

Diet
Standardized by the clinical unit

Each subject will have a participation of 64 hours approximately (approximately 43 hours confined with outpatient follow-up for the 36 and 48-h samples) for each of the periods, with a wash-out period of 35 days, starting as per the drug's intake in the 1st period, according to the following schedule:

4.4.4. Schedule

Pre-clinical Stage	Clinical Stage			Post-Clinical Stage
Activity form day -120 to 0	**Day**	**Time**	**Activity**	**Activity as per day 38**
▪ Protocol's elaboration. ▪ Preparation of the non-pregnancy letter, summary, schedule, ICF, and CRF. ▪ Protocol's approval. ▪ Approval by the Ethics and Investigation Committee and Investigation Committee. ▪ Filing at the COFEPRIS. ▪ COFEPRIS approval. ▪ Drug's receipt. ▪ Planning. ▪ Subjects screening.	**0**	As per 15:00 h	Admission.	▪ Subjects' clinical follow-up. ▪ CRF close-out. ▪ Delivery to the Analytical Unit. ▪ Clinical Report elaboration. ▪ Analytical stage. ▪ Final report. ▪ Study close-out. ▪ Approval request for the biological samples disposal.
	VISIT 1			
	01	06:00 h	Intake of 250 mL of water.	
		06:30 h	Catheter insertion.	
		07:30 - 07:55 h	Pre-dose sampling.	
		08:00 h	Drug intake.	
		08:30 h	Start of sampling (post-dose)	
	02	08:00 h	24.0-h sampling	
		Approximately at 10:00 h	Discharge from the Clinical Unit	
		20:00 h	36.0-h ambulatory sampling	
	03	08:00 h	48.0-h ambulatory sampling	
		Approximately 10:00 h	Temporal discharge, open appointment	
	Wash-out Period – 35 days			
	35	As per 15:00 h	Admission	
	VISIT 2			
	36	06:00 h	Intake of 250 mL of water	
		06:30 h	Catheter insertion	
		07:30 – 07:55 h	Pre-dose sampling	
		08:00 h	Drug intake	
		08:30 h	Sampling start (post-dose)	
	37	08:00 h	24.0-h sampling	
		Approximately at 10:00 h	Discharge from the Clinical Unit	
		20:00 h	36.0-h ambulatory sampling	
	38	08:00 h	48.0-h ambulatory sampling	
		Approximately at 10:00 h	Definitive discharge, open appointment.	

4.4.5. Investigational Product Identity

4.4.5.1. Packaging and labelling

Reference Eutirox® (approved formulation) from Merck, S.A. de C.V.

The reference drug will be supplied by Merck, S. A. de C. V. in its commercial presentation (original primary and secondary packaging).

The drug will be kept in an identified closed package with an external label containing the study code and, if applicable, an internal label stating as minimum the study code, batch number and expiry date. The drug will be stored according the product's requirements.

The reference drug will be given orally at a dose of 600 µg (3 tablets of 200 µg) of levothyroxine sodium, with 250 mL of water. Single dose.

Test Eutirox® (new formulation) from Merck, S.A. de C.V.

Test drug will be supplied by Merck, S. A. de C. V. in its commercial presentation (original or prototype primary and secondary packaging).

The drug will be kept in an identified closed package with an external label stating the study code and, if applicable, an internal label stating as minimum the study code, batch number and expiry date. The drug will be stored according to the product's requirements.

The test drug will be given orally at a dose of 600 µg (3 tablets of 200 µg) of levothyroxine sodium, with 250 mL of water. Single dose.

4.4.5.2. Storage

The drug should be kept safely, in a dry place, locked, at a temperature not higher than 25 °C, according to the label information.

4.4.5.3. Supply

Test and reference drugs will be received at thePPD . by the person in charge of the pharmacy or designated personnel according to the standard operating procedures, code: PPD
Once the drugs have been identified, they will be kept by the Clinical Unit until its dosing to the subjects.

Acceptance criteria for the test and reference drugs.

- Complete documentation:
  - ✓ Copy of the certificate of analysis for the test and reference drugs, endorsed by the Sponsor's Sanitary Officer, including the following tests:
    - Dose uniformity per content uniformity.
    - Evaluation.
    - Dissolution (if applicable).

- ✓ Letter issued by the Sponsor stating that the test and reference drug's batch fulfill with the current NOM-059-SSA1, endorsed by the Sanitary Officer.
- ✓ Under oath letter, endorsed by the Sponsor's Sanitary Officer, stating that the test batch for interchangeability test corresponds to the qualitative-quantitative formula for which the marketing approval will be applied for.

- Test drug with a label which fulfills with the minimum required by the current NOM-072-SSA1Labelling of Drugs and Herbal Remedies.
- Enough quantity of the test and reference drug.
- Valid expiry date during study.
- Test and reference drug in good physical conditions.

Rejection criteria for test and reference drugs:

- For blisters, that have been modified.
- If drug is receipt out of the storage or transportation conditions.
- If the primary packaging is damaged.
- If difference in review between of the test and reference drugs is > 5 %.

4.4.5.4. Accountability

The Sponsor will send enough quantity of the tests and reference drug to the PPD , so the study can be conducted and keep another complete set of reference and test drug as «retention samples». The person in charge of the pharmacy at the PPD or designated personnel will be in charge of dispensing the required doses of this on the study days and to keep an inventory of the used and stored doses as retention samples, according to the standard operating procedure, code: PPD

4.4.5.5. Retention samples

The unused drug will be kept at the retention samples warehouse, in its primary and secondary package, and locked for a 1 year period as per the study completion or until the expiry date (if it is earlier). The person in charge of the pharmacy o designated personnel, the PPD will be in charge of the custody of the retention samples.

4.4.5.6. Final Disposition

Following one year of the study completion or once the expiry date of the drugs is reached, these can be returned to the study Sponsor or will be made available for destruction by an authorized company, according to the standard operating procedure, code: PPD

4.4.6. Subjects Assignment Method to the Treatment Sequence

Once the inclusion criteria have been confirmed, a consecutive number will be assigned in ascending order from number 1 to the last case (44). A sequence A-B or B-A will correspond to each of these numbers from a randomization list previously produced by means of the software www.randomization.com and according to the standard operating procedure, code: PPD

4.4.7. Dose Selection in the Study

Since levothyroxine is an endogenous compound, a high ratio of the exogenous levothyroxine concentrations with regards to the baseline concentrations of the endogenous levothyroxine should be ensured to differentiate the real differences between the formulations. Therefore, use of doses over the therapeutic doses of levothyroxine is recommended. Recommended dose is equivalent to 600 µg and, therefore, it will be the dose used in this study. Three tablets of 200 µg, single dose will be given from test (B) and reference (A) product as corresponds to the period.

4.4.8. Selection and Timing of Dosing for Each Subject

Following the standardized fasting period, the drug will be given to all subjects from 08:00 to 08:08 h, in 4 groups of 9 subjects and 1 group of 8, with a 2-minute gap between each other for the dosing and each one of the activities. Groups distribution can be changed due to operation issues, if there is no impact in the study results.

The distribution of groups, subjects and timings of doses is shown in Table No. 4.

TABLE No. 4

Drug Dosing Schedule

Group	No. of Subjects	Dosing Time
A	1 - 9	08:00 h
B	10 - 18	08:02 h
C	19 - 27	08:04 h
D	28 - 36	08:06 h
E	37 - 44	08:08 h

4.4.9. Blinding

Randomization codes to the dosing sequence will be kept by the Principal Investigator. The Analytical Unit, the person in charge of the biological samples processing will be blinded during the samples analysis according to the standard operating procedures.

4.4.10. Previous and Concomitant Therapy

Soya products can reduce the intestinal absorption of levothyroxine, so it should be avoided.

Occasional use of paracetamol will be permitted within the screening period. The investigator may allow the subject to take paracetamol in case of pain during the trial, but the dose should not exceed 1000 mg/day for a maximum of three consecutive days. The amount of paracetamol administered must be recorded in the Case Report Format. Any additional concomitant therapy that becomes necessary during the trial from the date of signature of informed consent and any change to concomitant drugs must be recorded in the corresponding section of Case Report Format and in that case, the permanence of the subject in the study will be evaluated by the principal investigator.

The following are known interactions of levothyroxine, for the purposes of this clinical study, the use of concomitant medications (except paracetamol) is not permitted.

- Digitalis preparations: If levothyroxine therapy is initiated in digitalised patients, the dose of digitalis may require adjustment. Hyperthyroid patients may need their digoxin dosage gradually increased as treatment proceeds because initially patients are relatively sensitive to digoxin.

- Sympathomimetic agents: The effects of sympathomimetic agents (e.g. adrenaline) are enhanced.

- Protease inhibitors: Levothyroxine has been reported to lose its therapeutic effect when co administered with lopinavir/ritonavir.

- Antidiabetic agents: Levothyroxine may reduce the antihyperglycaemic effect of antidiabetics.

- Coumarin derivatives: Levothyroxine may potentiate the effect of coumarin derivatives due to plasma protein binding displacement.

- Ion exchange resins: Ion exchange resins, such as cholestyramine, colestipol, calcium salts and sodium salts of polystyrene sulphonic acid, inhibit the absorption of levothyroxine and should therefore not be administered within 4 to 5 hours of taking Levothyroxine sodium.

- Gastric acid binding agents containing aluminum, medications containing iron, calcium carbonate: Absorption of levothyroxine can be reduced by concomitant intake of gastric acid binding agents containing aluminum (antacids, sucralfate), medications containing iron and calcium carbonate. Levothyroxine sodium should therefore be taken at least two hours before these medications.

- Propylthiouracil, glucocorticoids and beta receptor blockers: These substances inhibit conversion of T4 to T3.

- Amiodarone inhibits the peripheral conversion of T4 to T3. Due to its high iodine content amiodarone can trigger hyperthyroidism as well as hypothyroidism. Particular caution is advised in the case of nodular goitre with possibly unrecognized autonomy.

- Salicylates, dicoumarol, furosemide, clofibrate, phenytoin: Levothyroxine may be displaced from plasma protein binding by salicylates, dicumarol, high doses (250 mg) of furosemide, clofibrate, phenytoin and other substances

- Oestrogen based contraceptives, medications used in postmenopausal hormone replacement: Levothyroxine requirements may increase during intake of oestrogen based contraceptives or during postmenopausal hormone replacement therapy

- Sertraline, chloroquine/proguanil: These substances reduce the efficacy of levothyroxine and increase the serum TSH level

- Tricyclic antidepressants Levothyroxine increases receptor sensitivity to catecholamines thus accelerating the response to tricyclic antidepressants (e.g. amitriptyline, imipramine)

4.4.11. Therapy Compliance

Throughout the period of administration of the dose, the complete taking of IMP will be checked and the intake time will be registered in the appropriate forms for this purpose.

A subject is compliant with the therapy if completes the two study periods.

4.5. Study Variables

Study variables will be divided in the following groups:

- Demography
- Pharmacokinetic
- Safety
- Biosafety; and
- Biological samples control.

4.5.1. Demographic Variables

- Age
- Sex
- Weight
- Height
- Body Mass Index

4.5.2. Pharmacokinetic Variables

To determine the SC panel *versus* time and the pharmacokinetic variables: AUC_{0-t} and C_{max}, 26 samples will be taken per period and approximately 10 mL of venous blood via catheter in the following times: -0.50, -0.25, 0.00 pre-dose and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.25, 3.5, 3.75, 4.0, 4.25, 4.5, 4.75, 5.0, 5.5, 6.0, 6.5, 8.0, 10.0, 12.0, 24.0 36.0 and 48.0 hours following the treatment and levothyroxine serum concentration will be determined.

Serum from these subjects will be collected as described in section 4.5.7.1.1. Dosing sequence and blood samples among subjects should be kept so that timing of these activities is the same among the subjects. Sampling exact time should be recorded in the appropriate forms.

Once each blood sample is taken, these will be sent to the samples processing area and serum will be obtained by centrifuging (between 30 and 60 minutes after sample collection). Serum samples should be frozen and kept at a temperature ≤ -40 °C until their shipment to the Analytical Unit and until analysis.

4.5.3. Safety Variables

Safety variables to be collected from subjects include:

- Complete medical history, including drugs and use of tobacco and alcohol.
- Clinical laboratory tests profile and thyroid profile.
- Electrocardiogram (in the selection and in the admission to each period).
- Vital signs.
- Adverse events.
- Abuse drug screening tests (at screening and each admission of the two periods).
- Pregnancy test (qualitative and quantitative) at screening and each admission.

4.5.4. Biosafety Variables

Safety for the personnel who handles the biological samples at the Clinical Unit and at the Analytical Unit.

- Screening for Hepatitis B Virus, Hepatitis C Virus, VDRL test (syphilis) and HIV (AIDS) at Screening only.

4.5.5. Variable Assessment Periods

For the study conduct the following periods have been planned:

- **Screening:** within the 30 days prior to the dosing.
- **First period of the study:** from the 16.00 hours prior to the first dose and at least 48.00 hours post-dose.
- **Wash-out period:** 35 days between each dose.
- **Second period of the study:** from the 16.00 hours prior to the second dose and at least 48.00 hours post-dose.
- **Study withdrawal:** following the 48.00-hour sampling in the second period.

- **Follow-up of adverse events:** adverse events occurring in a 14-day period following the dosing in the second period will be reported. Adverse events that were presented during the follow-up period and continued unresolved in the final visit, should be followed until resolved are no longer considered as clinically relevant or can be explained.
- **Final Visit:** 14 days after last administration of the study product.
- **End of study:** The study will end when the last subject has been subjected to the activities described in the final visit (section 4.4.2.6.) and the laboratory or cabinet findings or adverse events, if detected, have been explained and closed by the investigator.

For the wash-out period - drug's elimination - time when the subjects are out of the clinic, general directions will be given to each subject. They include specific information about feeding, restriction of drugs and overall care.

4.5.6. Control of Drugs and Liquid Intake

During the study conduct subjects should fast for at least 10 hours before the dosing and at least 4 hours following the dosing. Water is allowed ad libitum, except for the period between 1-hour pre-dose and the first meal (4 h after dose).

During confinement period, food will be provided as stated in Table No. 5. Forty-five minutes will be granted as maximum to have food; water intake is not subject to this period.

TABLE No. 5

Schedule of Feeding

FOOD	DAYS 0 and 35	DAYS 1 and 36
Breakfast	-----	As per 12:00 h
Lunch	-----	As per 16:00 h
Dinner	21:00 h	As per 21:00 h

There will be a 2-minute gap between groups (following the dose) for the food delivery according to the order and interval of the dosing.

4.5.7. Pre-analytical control for biological samples

To determine serum drug's concentrations, 26 samples of 10 mL approximately of venous blood will be taken per period by using catheter or venipuncture: -0.50, -0.25, 0.00 pre-dose and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.25, 3.5, 3.75, 4.0, 4.25, 4.5, 4.75, 5.0, 5.5, 6.0, 6.5, 8.0, 10.0, 12.0, 24.0 36.0 and 48.0 hours following the treatment.

4.5.7.1. Biological Fluids for the Study

4.5.7.1.1. Blood Samples

- According to the scheduled times, 26 blood samples will be taken by period, in test tubes specifically designed for venipuncture.
- During the clinical phase each sample volume will be of 10 mL. Blood withdrawn to clean the catheter should be discarded before the blood sampling (purge). Volume to be withdrawn by visit will be of 260 mL approximately, 520 mL of blood in total at the end of the study. This amount can slightly more due to the blood withdrawn for purging (approximately 0.5 to 1 mL) which may change. In addition to this amount of blood, approximately 20 ml of blood and approximately 20 ml of urine (laboratory test) are required for the selection. In women, consider 3 ml more for the pregnancy test. For the examination at the final visit consider the same amount.
- Real time of each sampling will be recorded.
- Each blood sample collected for pharmacokinetic analysis should be stored at room temperature for 30 - 60 minutes to allow for coagulation to occur, following this time period samples will be centrifuged at 2500 x g for **5** minutes at 4 °C (± 2 °C) to produce serum samples (If blood samples are not processed within 60 minutes of sample collection, the blood samples should be stored refrigerated prior to processing).
- Resulting serum samples will be deposited into 3 cryotubes containing approximately 1.5 - 2 mL of serum per tube.
- Serum tubes will be properly identified by the study number (##), case number (C##), sample number (S##), and visit number (V#), according to the standard operating procedures, code: PPD ; and they will be kept frozen at a temperature ≤ -40 °C until their delivery to the analytical unit.

4.5.7.1.2. Samples shipment

To ensure that the shipping conditions from the laboratory where the biological samples were processed for their freezing and storage to the analytical unit do not affect the samples stability, it will be essential to fulfill the following guidelines:

- The Principal Investigator or designated personnel will define the date and time of shipping in agreement with the analytical unit, as well as the transportation to be used and the approximate time for the transportation.
- Mechanism of control and security for the sample shipment will be defined by the person in charge of the shipment in agreement with the Principal Investigator or designated personnel.
- The person in charge of the samples processing will sign the material list attached, with a detailed description of the number of tubes and codes for each subject.

- An internal temperature reading system should be available in the container. A reading with its appropriate record will be made at least every 15 minutes.
- Additionally, the reading at the moment the material is leaving the samples processing area will be recorded, as well as at the moment it is received at the analytical unit.
- Samples transportation is the responsibility of the Clinical Unit where they were collected and processed.
- A member of the clinical unit will make the delivery.
- Samples must be frozen when delivered (-20 °C as maximum) when the container bags are checked.

4.5.7.1.3. Receipt, Inspection and Storage of Samples at the Analytical Unit

- The study responsible or the designated personnel at the analytical unit will receive the samples (with their documentation) and will review 100 % with the assistance of analysts and Quality Assurance, and will make notes in the appropriate form. Once the inspection is finished, the study responsible or designated personnel will store the samples in a deep freezer, and will document it on the equipment log.
- Quality Assurance will check the accuracy and reliability of the operations.

Samples failing the acceptance criteria will be rejected. In addition, those samples which do not have the appropriate documentation will be reported to the Clinical Unit stating the reason why those samples were rejected. Rejected biological samples will be kept at the Analytical Unit meanwhile their disposition is determined by agreement with the Analytical Unit and the Clinical Unit.

4.5.7.1.4. Biological samples handling by the Analytical Unit

Biological samples will be handled and processed as described in the appropriate analytical technique and according to the standard operating procedure, code: PPD

4.5.7.1.5. Disposal of biological samples

Following 30 calendar days to the final report issue, samples will be kept on the deep freezer and stored deep frozen, until they are recollected for their final disposition by an authorized company for that purpose.

4.5.7.1.6. Labelling of the deep-frozen samples

Cryotubes will be identified with labels according to the standard operating procedure, code: PPD . Each cryotube will show the study number, subject's number, period, and sample number specified in the protocol. Labels will be digitally designed and printed with a thermal printer. Type of label and printing will guarantee that the printing and adhesiveness resist several freezing and thawing cycles.

4.6. Quality Management

The quality assurance will be made by following the standard operating procedures, codes: PPD

The Quality Assurance Area follows up the study conduct process by means of quality control monitoring to check the compliance with the clinical protocol, internal standard operating procedures and the applicable guidelines of the Good Clinical Practice (ICH E6R1). Lack of compliance findings or opportunities for improvement as per the guidelines for the standard operating procedures, code: PPD will be followed-up. All information collected will be analyzed for the Quality Assurance report elaboration and issue.

4.7. Documentation

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

4.8. Direct Access to the Source Data

As part of the Sponsor's responsibilities, the study can be monitored in the analytical and clinical aspects to ensure data accuracy and understandability by accessing to the source documents to verify the Case Report Forms.

4.9. Agreement with an Ambulance and Hospital Service for Emergencies

For adverse events, the medical staff will provide the immediate medical assistance required according to the adverse event occurred. In case of a serious event which requires hospital assistance, the Principal Investigator or medical staff on duty will indicate the transfer of the subject to the agreed hospital and will request the external ambulance service.

5. Ethical and Legal Aspects

5.1. Ethics and Investigation Committee and Investigation Committee

Before the start of the study, written approval from the Ethics and Investigation Committee and from the Investigation Committee, which should provide a list of their members, as per request of the study Sponsor or Principal Investigator. In the same way, is necessary the written authorization of the health authority in Mexico through the COFEPRIS before starting with any procedure of the study. If necessary due to modifications to the clinical protocol, case report form or informed consent form, a new approval from both committees and COFEPRIS will be obtained.

5.2. Ethical and Regulatory Compliance

This protocol fulfills with the current regulations on clinical research, including: the Guidelines of the Good Clinical Practice (GCP), Ethical Principles for Medical research in human beings from the Declaration of Helsinki issued by the 64th General Assembly of the World Health Organization, Fortaleza, Brazil, October 2013; the General Health Act and the Regulations of the General Health Act in the Field of Health Research, as well as the International Ethical Guidelines for Health-related Research Involving Humans, Prepared by the Council for International Organizations of Medical Sciences (CIOMS) in collaboration with the World Health Organization (WHO), Geneva 2016.

5.3. Approval by Regulatory Authorities

This study will be carried out according to the provisions of the regulations set forth in the NOM-177-SSA1-2013 which states the tests and procedures for proving that a drug is interchangeable and the requirements which third parties performing interchangeability tests should fulfill; the requirements for conducting biocomparability studies; requirements for authorized third parties, research centers or hospital institutions conducting biocomparability tests, as well as the NOM-012-SSA3-2012 which states the criteria for conducting research project for human beings health.

5.4. Informed Consent Form and Non-Pregnancy Commitment Letter

The Principal Investigator or designated person, will inform the subjects about the appropriate aspects of his/her participation in this study. The process for the informed consent obtainment form the subject will be conducted according to all applicable regulatory requirements. The person obtaining the consent, witnesses and the subject should sign and date the informed consent form before performing any study related procedures. Decision regarding the subject's participation in the study, is absolutely free. The Principal Investigator or the person who gets the consent should stress to the subject that his/her consent regarding his/her participation in the study, can be withdrawn at any time without any prejudice or loss of benefits which he/she is entitled anyway.

Child-bearing potential women will have to sign and date a Non-Pregnancy Commitment Letter where they state that they are not pregnant or have any delay in their cycle, that they will avoid getting pregnant by means of barrier or non-hormonal contraceptive methods throughout the study.

If the informed consent form or the Non-Pregnancy Commitment Letter are modified during the study, the Principal Investigator should fulfill all the applicable regulatory requirements necessary for the approval of the informed consent form amendment by the Ethics and Investigation Committee and the Investigation Committee and use the amended form in the study.

5.5. Confidentiality, responsibility and compensation

Information collected in this study will be exchanges exclusively between Merck, S.A. de C.V. and the PPD and will be treated as confidential.

Subjects will sign a consent specifying that they will receive an economical compensation for their participation in the study. The Sponsor will pay (o compensate, if applicable) the resulting injuries or diseases caused by the subjects' participation in the study until their resolution according to the medical opinion. The Sponsor will not pay if this is due to the subject's negligence, irresponsible conduct or medical conditions no related to the study.

The Ministry of Health, the Ethics and Investigation Committee and the Investigation Committee will be the only authorized bodies for reviewing the study documents (including the participant subjects' identity data) and the documents considered confidential by the PPD and Merck, S. A. de C. V.

Study participant subjects' personal data will be confidential and will be protected by the Federal Law on the Protection of Personal Data held by Private Parties, which is stated in our PRIVACY NOTICE and can be checked in our website www.cecype.com.

6. Samples Chemical Analysis

Levothyroxine (T4) serum concentrations will be determined by liquid chromatography coupled in a mass detector/mass according to the physicochemical properties and reported in the international scientific literature (LC MS/MS), which will be validated prior to the start of the study according to the applicable guidelines and the study working plan, as well as to the applicable analytical technique, in compliance with the applicable Analytical Unit and Quality Assurance procedures, and according to the NOM-177-SSA1-2013 and the Good Laboratory Practice. The analytical method details will be described in the validation protocol.

7. Sample Size Determination, Pharmacokinetic and Statistical Analysis

The pharmacokinetic and statistical analysis for the levothyroxine serum concentrations will be carried at the PPD as per its standard operating procedures and in compliance with the NOM-177-SSA1-2013.

7.1. Sample Size Determination

The sample size determination was based on the variability seen in the most recent study conducted by the study Sponsor EMR200125-001 (comparative study of the levothyroxine formulations for approval in Europe) where the intrasubject variability for AUC_{0-72h} was 23.7 % and the C_{max} was 17.7 %. Using the most variable parameter AUC_{0-72h}, a difference of 5 %, a power of 90 % and applying the accepted bioequivalence range of 80 % to 125 %.

By using the Chow & Wang equation, and substituting the aforementioned values, a sample size of 34 subjects was obtained. If a drop rate of 20 % is considered, the sample size should be of 44 subjects.

7.2. Pharmacokinetic Analysis

The pharmacokinetic analysis will be obtained by using a non-compartmental analysis with the use of the current versions of the Phoenix® WinNonlin® software.

The following parameters will be determined. They will be calculated with the uncorrected data of the baseline levels (raw values), as well as the corrected values by baseline levels (adjusted values). The mean of the pre-dose levothyroxine levels should be used for the baseline adjustment of the post-dose levels. Any negative values obtained from baseline correction should be designated as zero (0):

C_{max}: maximum serum concentration observed directly from the concentration-time curve.

$C_{max(aj)}$: maximum serum concentration in pre dose corrected data.

t_{max}: time when the maximum serum concentration was obtained.

AUC_{0-t}: area under the curve of the serum concentration as a function of time, from time 0 until the last sampling time by means of the trapezoidal rule.

$AUC_{0-t\ (aj)}$: area under the curve of the serum concentration as function of time, from time 0 until the last sampling, by means of the trapezoidal rule in pre dose corrected data.

$AUC_{0-\infty\ (aj)} = AUC_{0-t\ (aj)} + C_{t\ (aj)} / K_{el}$

K_{el}: elimination constant, calculated from the semi-logarithmic graphic of the serum concentration as function of time. It is estimated by means of a simple linear regression analysis from at least the last three serum concentrations different to zero.

$t_{1/2}$: time of the half-life or elimination half-life calculated as $Ln\ (2)/K_{el}$

7.3. Statistical Analysis

7.3.1. Descriptive Statistics

Adjusted concentrations will be tabulated by treatment and by time of all subjects, stating the drug or formulation, the arithmetic mean, standard deviation and variation coefficient.

A descriptive statistic will be presented by the adjusted serum concentrations, by treatment globally (arithmetic mean, minimum value, maximum value, standard deviation and coefficient of variation).

The descriptive statistic will be determined from the individual adjusted pharmacokinetic parameters, by treatment, stating the period, sequence, quotient of the reference test, as well as its logarithm for the pharmacokinetic parameters used to determine the bioequivalence (geometric means, arithmetic mean, median, minimum value, maximum value, standard deviation and variation coefficient). This will only apply for evaluable subjects.

An evaluable subject will be one who has completed both periods of the study and has at least 60% of their samples with measurable concentrations, who has not presented vomiting or diarrhea before 2 times the median of the t_{max} or 2 times the value of t_{max} and who has not presented any situation that could modify the result of the study due to uncontrolled variables.

A graphic of the adjusted serum concentration against individual and average time in the arithmetic and semi-logarithmic scale.

7.3.2. Statistical Analysis for Bioequivalence

7.3.2.1. Variance Analysis (ANADEVA)

The general linear model of the analysis of variance that represents the experimental design for the analysis of data of the adjusted pharmacokinetic variables will be used, considering the following sources of variation in an additive way:

- Dosing sequence.
- Nested subjects in the dosing sequence call inter-subject variability or inter-subject residual variability.
- Dosing period.
- Therapy or formulation.
- Experimental error, called intra-subject variability or intra-subject residual variability.

This general lineal model, applied to the crossed designs to determine bioequivalence is the following:

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

Where:

μ = general mean.

G_k = fixed effect of k^{th} sequence.

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

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

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

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

The sequence effect should be assessed using the square means of the nested subject in the sequence as a term of error. All other main effects should be assessed against the residual error (error square mean) and F values reported as appropriate. Additionally, it should be stated if the source of variation is significant when p is < 0.05 ($p < 0.05$).

In the event of a significant effect in the variability factors for the sequence or period, actions will be taken in accordance with the NOM-177-SSA1-2013.

7.3.2.2. Hypothesis Statement for the double, one-sided Schuirmann's t test,

The double, one-sided t test (Schuirmann) will be done with the obtained data from the logarithmic transformation for the $AUC_{0\text{-}t\ (aj)}$ and $C_{max\ (aj)}$, by means of the 90 % CI construction for the quotient between the average pharmacokinetic parameters logarithmically transformed from the test and reference drugs.

$$H_0: \mu_p / \mu_r \leq Li \text{ or } \mu_p / \mu_r \geq Ls$$

$$H_1: Li < \mu_p / \mu_r < Ls$$

Null hypothesis for bioequivalence is rejected if Schuirmann's P probability P(t) for the lower limit (*Li = 0.8*) and upper (*Ls = 1.25*) is $P < 0.05$.

7.3.2.3. Determination of the Classical 90 % confidence Interval

Compliance with the *Guide to Establish Interchangeability of Oral Solid Drugs containing Levothyroxine Sodium* (COFEPRIS, 2017). The pharmacokinetic parameters that will be used for the statistical analysis of bioequivalence are $C_{max\ (aj)}$ and $AUC_{0\text{-}t\ (aj)}$ of levothyroxine (T4), by means of 90 % confidence intervals for the geometric means of these pharmacokinetic parameters to check their bioequivalence. The classical 90 % confidence interval will be calculated by means of the following equation for the lower and upper limit, respectively.

$$\left[\exp\left(\overline{Y}_T-\overline{Y}_R-t_{1-\alpha,n_1+n_2-2}\hat{\sigma}_W\sqrt{\frac{1}{2}\left(\frac{1}{n_1}+\frac{1}{n_2}\right)}\right),\exp\left(\overline{Y}_T-\overline{Y}_R+t_{1-\alpha,n_1+n_2-2}\hat{\sigma}_W\sqrt{\frac{1}{2}\left(\frac{1}{n_1}+\frac{1}{n_2}\right)}\right)\right]$$

Where:

Y_T = population media for the assessed pharmacokinetic parameter of the test drug.
Y_R = population media of the assessed pharmacokinetic parameters of the reference drug.
σ_w = square root of the square mean for the error of the assessed pharmacokinetic parameter.
$t_{(1-\alpha,N-2)}$ = distribution of the Student's t with an α probability of 0.05 and N-2 degrees of freedom (df) = N – 2 with an α probability = 0.1
n_1 = number of volunteers in the sequence 1.
n_2 = number of volunteers in the sequence 2.

The statistical analysis to show interchangeability will be done using only the corrected values of the parameters for $C_{max\ (aj)}$ and $AUC_{0\text{-}t\ (aj)}$, equivalence margins will be considered as 80 % - 125 %.

7.3.3. Outliers

An analysis to identify the outliers (extreme values) based on the estimation of the Student intra-subject residual values will be done using Excel software. By means of the determination of the residual value obtained with the model using the following equation:

$$\widehat{e_{ijk}} = Y_{ijk} - \hat{Y}_{ijk}$$

Where:

Y_{ijk} is the observed response.

$\hat{Y}_{ijk}$ is the estimated value with the model.

This residual value should be in the Student fashion by using the following equation:

$$e_{jklstd} = \frac{\widehat{e_{ijk}}}{\sqrt{\left(\frac{S-1}{2*S}*CMerror\right)}}$$

Where:

$\widehat{e_{ijk}}$ is the residual value previously determined.

S is the number of subjects considered for the assessment

CMerror, value of the mean square value of error, corresponding to each of the assessed pharmacokinetic parameters.

Determination of outliers will be done for the pharmacokinetic parameters of C_{max}, and $AUC_{0\text{-}t}$ for both analytes.

Criterion: data which extent is higher than ± 2 intra-subject standardized residual values will be considered outlier (extreme).

7.3.4. Deviation to the Statistical Plan

The study statistical plan will correspond to what is specified in the section 7.3 of this protocol with regards to the methods and criteria for the statistical tests thus established to compare the bioavailability of the study drug.

All deviations should be justified by statistical or scientific evidence and any change should be reported in the original statistical plan, in the study master file and in the statistical pharmacokinetic report and study final report.

Subject data will not be replaced and any missing data will be considered as non-existent data. Likewise, data cannot be removed from the statistical analysis, except for the following cases:

- Elimination of date due to vomiting and diarrhea.

Data from the research subjects who suffer from vomiting and diarrhea during the bioequivalence study for immediate release products can be removed from the statistical analysis if vomiting or diarrhea occurs before twice the median for t_{max} or twice the value for t_{max} from the research subject in a given period.

- Research subject with very low serum concentrations for the study drugs.

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

A research subject is considered to have very low concentrations, if the $AUC_{(aj)}$ is < 5 % for the geometric mean for the AUC of the reference drug (it should be calculated without including outliers). Exclusion of data for this reason, will only be accepted under scientific rationale and previous review of the case by the COFEPRIS.

- Finally, all data which do not show significant outliers will be considered according to the criteria for outliers in the section 7.3.3 of this protocol. Outliers exclusion should be supported by scientific evidence.

7.4. Biowaiver of bioequivalence test for other strengths

According to the official Mexican norm (NOM-177-SSA1-2013) if a product has more than one strength, in the same pharmaceutical form and shows acceptable in vitro dissolution testing of all strengths, a biowaiver is possible if the following requirements are met:

The proportionality (constant relationship) in the content of drug (s) and additive (s) in the qualitative-quantitative formula with respect to the drug was found. Proportionality must meet the following criteria (NOM-177-SSA1-2013):

- The drug (s) or additive (s) are in the same proportion between the different concentrations.
- The drug (s) or additive (s) are not exactly in the same proportion between the different doses, but the ratio of active ingredients relative to the total weight of the dosage form can vary up to 10% without any impact on the dissolution rate of the drug.
- For potency drugs, where the amount thereof in the dosage form is very low (less than 10 mg per tablet), and the total weight of the dosage form is equal at all doses, they could be proportionality if don´t vary by more than 10% of the total weight of the dosage form.
- For immediate release, the coated components, colorings, flavorings and capsule shell should not be considered in the calculations to establish the proportionality of the formulas.
- The dissolution profile is similar between the bioequivalent strength of the test drug and the test drug with the strengths to exempt, which must be performed by an authorized third party.
- Sufficient scientific evidence is submitted to demonstrate that the drug exhibits linear pharmacokinetics in the range of concentrations applied.
- Manufacturing processes are validated.

8. Adverse Events

8.1. Warnings

Prior to the study drug's administration, information of the prescribing information related to the study side effects (approved by the Ministry of Health) should be considered. The Principal Investigator and the involved physicians in the study should know in advance the prescribing information.

8.2. Adverse Events Monitoring

Subjects should be carefully monitored with regards to the presence of adverse events. Adverse events should be established in terms of their seriousness, severity and causality with the study drug.

8.3. Observation Period for Adverse Events

Adverse events occurring from the signature of the informed consent form and until 14 days following the dosing in the second period of the study will be reported.

8.4. Adverse Events Definitions

8.4.1. Adverse Event

Any untoward medical event which might occur in a research subject during the clinical investigation stage of a drug or vaccine, but that not necessarily has a causality with it (NOM-220-SSA1-2016).

Adverse events related to the use of drugs in humans, whether related with it or not, include the following:

- An adverse occurring during the use of a drug in a professional setting.
- An adverse event occurring due to the abuse of drugs or illicit drugs.
- An adverse event occurring following the drug's intake stop.
- An adverse event where there is a reasonable possibility that the event occurred only as a result of the subject's participation in the study (e.g., serious or non-serious adverse event due to the antihypertensive drugs discontinuation during the wash-out phase) should be reported as an adverse event even if it is not related with the investigational product.
- Clinical onset of any failure of the expected pharmacological action.

If the event fulfills with the "serious" adverse event criteria should be recorded and reported as such.

8.4.2. Serious Adverse Event

A serious adverse event is any clinical onset occurring following the dosing of any drug, including vaccines, and that (NOM-220-SSA1-2016):

- Causes the subject's death.
- Is life-threatening for the subject when occurring.
- It is necessary to admit the subject at the hospital or extend his/her stay at the hospital.
- Causes permanent or significant inability or disability.
- Causes abnormalities or malformations in the newborn.
- Are considered medically important.

Some important medical events, even though they not produce death or are life-threatening, or require hospitalization, can be considered serious adverse events based on the appropriate medical opinion; or those which pose a risk for the subject, leading to medical or surgical intervention to avoid them. Example of those medical events include allergic bronchospasm which requires intensive treatment at the emergency room or at home; blood dyscrasias or seizures which do not lead the hospital admission or drug dependence or abuse.

Life-threatening means that the subject was, at the investigator's opinion, at immediate risk of death by the reaction when it occurred.

Disability means an important disorder in the person's ability to perform his/her daily activities.

8.4.3. Unexpected Adverse Reaction

An unexpected adverse reaction is an adverse reaction which nature or severity is not described in the product's prescribing information, nor in the documentation submitted for the marketing approval (NOM-220-SSA1-2016).

8.4.4. Exposure during pregnancy

If a female subject becomes pregnant during their participation in the study, the corresponding notification will be made as a serious adverse event. Follow-up will be mandatory throughout the pregnancy and until at least the first 6 months of life of the newborn (NOM-220-SSA1-2016).

Based on the subject's preference, follow-up can be done through telephone contact or medical consultation in the clinical unit, at least every two months during pregnancy and up to 6 months of life of the newborn or until the pregnancy is resolved (in case an abortion happens).

8.5. Causality of the Adverse Event with the Investigational Product

Establishment of the causality of the adverse events with the administered study drug is a clinical decision based on the available information when the adverse event occurred.

According to the causality assessment provided by the World Health Organization and the Uppsala's Monitoring Center, probabilistic characteristics are the following (NOM-220-SSA1-2016):

- **Certain:** clinical event, including laboratory tests abnormalities, which occur with a plausible temporary sequence with regards to the drug's dosing, and which cannot be explained by the concurrent disease, or by other drugs or substances. Drug's dechallenge response (withdrawal) should be clinically plausible. The event should be definitive from the pharmacological or phenomenological point of view, using, if necessary, the conclusive rechallenging procedure.
- **Probable:** clinical event, including laboratory tests abnormalities, which occur with a reasonable temporary sequence with regards to the drug's dosing and it is unlikely that it is attributed to the concurrent disease, or other drugs or substances; and that shows a clinically reasonable response to the drug's dechallenge. It is not necessary to have information about rechallenge to assign this definition.
- **Possible:** clinical event, including laboratory tests abnormalities, which occur with a reasonable temporary sequence with regards to the drug's dosing, but that can be explained by the concurrent disease, or by other drugs or substances. Information regarding the drug's dechallenge may be missing or unclear.
- **Unlikely:** clinical event, including laboratory tests abnormalities, which occur with an unlikely temporary sequence with regards to the drug's dosing which can be explained in a more plausible way by the concurrent disease, or by other drugs or substances.
- **Conditional or not classified:** clinical event, including laboratory tests abnormalities, reported as an adverse reaction, for which it is essential to get more data to make an appropriate assessment or which data are being analyzed.
- **Not evaluable or unclassifiable:** a report that suggests an adverse reaction which cannot be assessed due to insufficient or contradictory information and which data cannot be checked or completed.

8.6. Adverse Event Severity

Adverse events severity or seriousness should be classified as follows:

- Mild: occur with signs and symptoms easily tolerated that do not require treatment; they do not require hospitalization or its extension, and do not require that the causative drug is stopped.
- Moderate: interfere with daily activities (may cause work or school absence), without threatening the subject's life directly. They require pharmacological therapy and may not require that the causative drug is stopped.
- Severe: interfere with daily activities (may cause work or school absence). Require pharmacological therapy and the discontinuation of the causative drug.

8.7. Adverse Events Documentation and Report

Any adverse event occurring during the study period should be fully recorded both in the subject's medical chart and in the Case Report Form.

Documentation should be supported by a record in the subject's medical chart. Laboratory tests abnormalities considered as clinically relevant, for instance, which cause the subject's withdrawal from the study and require therapy or cause apparent clinical signs in the subject, or that are considered relevant by the investigator, should be reported as an adverse event. All adverse events should be described in detail, including the subject's identity (name, age and sex), adverse event, suspected drug, reporter data, onset date and event's term and therapy, generic and distinctive name, dosage, route of administration, reason of prescription, event's consequence and important data in the medical history.

The report of adverse events will be the responsibility of the PPD and will be made according to the standard operating procedure, code: PPD and the Mexican Official Standard for the Pharmacovigilance Setting Up and Operations in Mexico, NOM-220-SSA1-2016. Therefore, reports from institutions or establishment which intend to or conduct research in human beings should be coded with reported clinical onset using the current MedDRA term. Timelines for reports submission to the National Center of Pharmacovigilance (CNFV) from the Federal Commission for the Protection against Sanitary Risks (COFEPRIS) in the country and to the Sponsor are as follows:

- Serious adverse event: up to 7 calendar days.
- Non-serious adverse event: report at the end of the study.
- Two or more serious events, similar in the same location, with the same drug and batch number: immediately, no later than 48 hours.

Report to the CNFV should be made by the Pharmacovigilance Officer at the PPD in compliance with the NOM-220-SSA1-2016.

The ways to make the report to the Sponsor are the following:

PPD

PPD

Telephone:	PPD
Mobile:	PPD
E-mail:	PPD

According to the local law and regulations, adverse events should be reported to the appropriate Ethics and Investigation Committee, Investigation Committee and regulatory authorities.

9. Data Use and Publication

All data and results, and all rights and intellectual property for the data and results from this study will be property of Merck, S. A. de C. V. that can use the data in several ways, such as submitting them to government regulatory authorities or sending them to other investigators.

Although the Investigator is free to use the data obtained in the study for scientific purposes, he/she should discuss any publication with Merck, S. A. de C. V. in advance, and he/she should obtain the written consent from the Sponsor for the intended publication.

The Sponsor acknowledges the Investigator's right to publish the results once the study is completed. Anyway, the Investigator should send the paper's draft to be published or its summary to Merck, S. A. de C. V., 30 days in advance to the submission of the final version for publication.

This will be reviewed soon and approval will not be unnecessarily delayed. In case of disagreement between the Sponsor and the Investigators, publication content will be discussed to find an appropriate solution for both parties.

10. Management of Concurrent Events (Administrative and Medical)

10.1. Research Protocol Amendments

Research protocol amendments may be issued by the Principal Investigator in agreement with the Sponsor and vice versa, and should be approved by the Ethics and Investigation Committee and by the Investigation Committee. Administrative changes which do not affect the study design should be agreed and approved by the Sponsor, the Analytical Unit and the Principal Investigator. They should be submitted to the Ethics and Investigation Committee and to the Investigation Committee.

10.2. Deviations to the Protocol

Deviations to the research protocol occurring during the study conduct should be reported to the Principal Investigator, Study Sponsor, to the Ethics and Investigation Committee and to the Investigation Committee.

Whenever a deviation to the protocol occurs during the clinical stage conduct, it should be assessed by the Principal Investigator. If considering that the subject's continuation in the study may affect the study's results, the Sponsor will be informed; and jointly they will decide the continued subject's participation in the study.

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12. APPENDIXES

12.1. Schedule of Assessments

"A prospective, single dose, randomized, open, crossover, comparative study to establish bioequivalence in healthy subjects between the new formulation vs. the currently approved formulation of levothyroxine 3 tablets of 200 µg Eutirox® Merck, S.A. C.V."

Trial Day	SCR	ADMISSION	Period 1 TD-1	TD 1																								TD2	TD3	
			wash-out	TD1- TD35																										
			Period 2 TD34	TD 35																								TD36	TD37	EOT
Time Post-dose (h)			**Pre-dose: -0.50, -0.25**	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**2.75**	**3**	**3.25**	**3.5**	**3.75**	**4**	**4.25**	**4.5**	**4.75**	**5**	**5.5**	**6**	**6.5**	**8**	**10**	**12**	**24**	**48**	**TD51**
Informed Consent	X																													
Hospitalization [1]		←																									→			
Ambulatory Visits	X																												X	
Incl./Excl. Criteria [2]	X	X																												
Medical History	X																													
Body weight height [3]	X																													
Virus serology (HCV, HBV, HIV and VDRL)	X																													
Urine drugs-of-abuse & alcohol screens [4]	X	X																												
Serum Pregnancy Testing (WOCBP) [12]	X	X																												
Physical examination [5]	X	X																												
Vital Signs [6,7]	X	X	X	←																							→	X	X	
ECG 12-lead [6,7]	X	X																											X	
Safety Laboratory [12] (Hematology, Clinical Chemistry [13], Urinalysis)	X																												X	
Randomization [8]		X																												
IMP administration [9]					X																									
PK																														
PK			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Safety																														
Adverse Events [10]	X	X	X	←																							→			X
Concomitant medication [11]	X	X	←																								→			X
End of Clinical monitorin call																														X

Footnotes

[1] Hospitalization: From TD–1 until the 24 hours post-dose for each period

[2] Subject eligibility must be checked again on TD-1 prior to randomization and TD34 prior second administration.

[3] Height [cm] (screening) and weight [kg] (screening).

[4] Urine screen for drugs of abuse (amphetamines, benzodiazepines, cocaine, methamphetamines, morphine and tetrahydro – cannabinoids) and alcohol. The tests can be repeated at any time at the discretion of the investigator.

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

[6] Vital signs include systolic and diastolic blood pressure, pulse rate and body temperature. The TD1 and TD35 the vital signs will be taken pre-dose and then at each turn.

[7] For vital signs and ECG assessment, the subjects should be at rest and semi-supine for at least 5 minutes before recording and should remain resting and supine during recordings.

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

[9] Subjects will receive IMP after an overnight fast of at least 10 hours. The IMP will be administered at morning, 600 µg, single dose, with 250 mL of water (in compliance with the NOM-177-SSA1-2013). No food will be allowed for the next 4 hours, until a standardized lunch will be served.

[10] Spontaneous AEs will be continously reported since the subjects sign the informed consent until complete the clinical monitoring ; subjects will be asked specifically at admission in both period, pre dose, after each administration, during the hospitalization and when subjects attend to the clinic for the blood sampling.

[11] Subjects will be asked at selection, at admission in both period and when the subject complete the clinical monitoring,

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

[13] Blood sample collection for safety laboratory assessments will be performed after a fast of at least 10 hours.

12.2. Sampling Schedule

	Day	Activity	Scheduled time	Scheduled time
First Visit	Day 0	*ADMISSION*	16:00	-16.00
		DINNER	21:00	-11.00
		FASTING START AND REST	22:00	-10.00
	Day 01	*GROOMING AND INTAKE OF 250 ML OF WATER*	06:00	-2.00
		BLOOD SAMPLE 01 (Pre-dose)	07:30	-0.50
		BLOOD SAMPLE 02 (Pre-dose)	07:45	-0.25
		BLOOD SAMPLE 03 (Pre-dose)	07:55	-0.00
		DRUG'S DOSING	08:00	0.00
		BLOOD SAMPLE 04	08:30	0.50
		BLOOD SAMPLE 05	09:00	1.00
		BLOOD SAMPLE 06	09:30	1.50
		BLOOD SAMPLE 07	10:00	2.00
		BLOOD SAMPLE 08	10:30	2.50
		BLOOD SAMPLE 09	11:00	3.00
		BLOOD SAMPLE 10	11:15	3.25
		BLOOD SAMPLE 11	11:30	3.50
		BLOOD SAMPLE 12	11:45	3.75
		BLOOD SAMPLE 13	12:00	4.00
		BREAKFAST	12:00	4.00
		BLOOD SAMPLE 14	12:15	4.25
		BLOOD SAMPLE 15	12:30	4.50
		BLOOD SAMPLE 16	12:45	4.75
		BLOOD SAMPLE 17	13:00	5.00
		BLOOD SAMPLE 18	13:30	5.50
		BLOOD SAMPLE 19	14:00	6.00
		BLOOD SAMPLE 20	14:30	6.50
		BLOOD SAMPLE 21	16:00	8.00
		LUNCH	16:00	8.00
		BLOOD SAMPLE 22	18:00	10.00
		BLOOD SAMPLE 23	20:00	12.00
		DINNER	21:00	13.00
	Day 02	BLOOD SAMPLE 24	08:00	24.00
		BLOOD SAMPLE 25	20:00	36.00
	Day 03	BLOOD SAMPLE 26	08:00	48.00

	Day	Activity	Scheduled time	Scheduled time
Second Visit	Day 35	*ADMISSION*	16:00	-16.00
		DINNER	21:00	-11.00
		FASTING START AND REST	22:00	-10.00
	Day 36	*GROOMING AND INTAKE OF 250 ML OF WATER*	06:00	-2.00
		BLOOD SAMPLE 01 (Pre-dose)	07:30	-0.50
		BLOOD SAMPLE 02 (Pre-dose)	07:45	-0.25
		BLOOD SAMPLE 03 (Pre-dose)	07:55	-0.00
		DRUG'S DOSING	08:00	0.00
		BLOOD SAMPLE 04	08:30	0.50
		BLOOD SAMPLE 05	09:00	1.00
		BLOOD SAMPLE 06	09:30	1.50
		BLOOD SAMPLE 07	10:00	2.00
		BLOOD SAMPLE 08	10:30	2.50
		BLOOD SAMPLE 09	11:00	3.00
		BLOOD SAMPLE 10	11:15	3.25
		BLOOD SAMPLE 11	11:30	3.50
		BLOOD SAMPLE 12	11:45	3.75
		BLOOD SAMPLE 13	12:00	4.00
		BREAKFAST	12:00	4.00
		BLOOD SAMPLE 14	12:15	4.25
		BLOOD SAMPLE 15	12:30	4.50
		BLOOD SAMPLE 16	12:45	4.75
		BLOOD SAMPLE 17	13:00	5.00
		BLOOD SAMPLE 18	13:30	5.50
		BLOOD SAMPLE 19	14:00	6.00
		BLOOD SAMPLE 20	14:30	6.50
		BLOOD SAMPLE 21	16:00	8.00
		LUNCH	16:00	8.00
		BLOOD SAMPLE 22	18:00	10.00
		BLOOD SAMPLE 23	20:00	12.00
		DINNER	21:00	13.00
	Day 37	BLOOD SAMPLE 24	08:00	24.00
		BLOOD SAMPLE 25	20:00	36.00
	Day 38	BLOOD SAMPLE 26	08:00	48.00

12.3. Summary

Study Title	"Prospective, single dose, randomized, open label, comparative, cross-study to establish bioequivalence between the new formulation and the approved formulation for Levothyroxine (Eutirox® from Merck, S. A. de C. V.) given as 3 tablets of 200 µg p.o. in healthy volunteers"
Study Objective	To establish the bioequivalence between the new and approved levothyroxine's formulation in tablets of 200 µg (Eutirox® from Merck, S. A. de C. V.) in healthy volunteers for the marketing approval of the new formulation in all its strength. The lower strengths will be justified with the biowaiver criteria described in NOM-177-SSA1-2013..
Study Design	Experimental design, longitudinal, comparative, open-label, randomized, crossed in blocks, scheme A-B and B-A.
Rationale for the Study Design	As the goal is to prove the lack of significant differences in the pharmacokinetic parameters where each subject is his/her own control, the chosen experimental design is one of those suggested by the NOM-177-SSA1-2013, crossed, open label, two sequences and two periods of treatment (scheme A - B and B - A), and single dose. Section 8.4.8.1 of this Standard states that drugs with a long half-life, crossed or parallel designs should include a limited sampling for at least 72 hours. However, prior studies showed that sampling times longer than 48 hours do not allow to characterize differences between exogenous and endogenous levothyroxine concentrations with certainty. Therefore, bioequivalence studies for this drug should be truncated up to 48 hours (*Guidance to Establish the Interchangeability of Oral Solid Drugs Containing Levothyroxine Sodium, COFEPRIS, 2017).* According to the aforementioned, a 48-h sampling time, which is compliant with the Mexican health authorities' requirement, is proposed. In addition, it allows to characterize at least the 80 % of the AUC, by having 26 total samples and a wash-out period of 35 days between the two study periods.
Population	44 male and female healthy subjects.
Rationale for the Sample Size	The sample size determination was based on the variability seen in the most recent study conducted by the study Sponsor EMR200125-001 (comparative study of the levothyroxine formulations for approval in Europe) where the intrasubject variability for $AUC_{0\text{-}72h}$ was 23.7 % and the C_{max} was 17.7 %. Using the most variable parameter $AUC_{0\text{-}72h}$, a difference of 5 %, a power of 90 % and applying the accepted bioequivalence range of 80 % to 125 %, a sample size of 34 subjects was obtained. If a drop rate of 20 % is considered, the sample size should be of 44 subjects.
Dosage Form	Tablets 200 µg.
Dose	600 µg (3 tablets of 200 µg), orally, with a 10-h fasting state.
Pre-treatment	Not required.
Wash-out Time	35 days.
Clinical Laboratory Tests	Complete blood count; 4-panel chemistry; urinalysis; liver function tests; HIV, hepatitis B and C, and VDRL test; and qualitative urine test for illicit drugs, and pregnancy test for women. Thyroid profile.
Reference Product	Eutirox® from Merck, S. A. de C. V. (approved formulation).
Test Drug	Eutirox® from Merck, S.A. de C.V. (new formulation)
Endpoints	48.00-h follow-up to collect 26 blood samples to determine levothyroxine (T4) in serum and to calculate the $AUC_{0\text{-}t}$ and C_{max} corrected values.
Sampling Times	-0.50, -0.25, 0.00 pre-dose and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.25, 3.5, 3.75, 4.0, 4.25, 4.5, 4.75, 5.0, 5.5, 6.0, 6.5, 8.0, 10.0, 12.0, 24.0 36.0 and 48.0 h post-dose.
Special Remarks	Pre-dose sampling will be carried out. Statistical analysis to prove interchangeability will only be made using the corrected values for the parameters C_{max} and $AUC_{0\text{-}t}$. Equivalence margins will be considered as 80 % - 125 %.

12.4. Diet

Diet will be provided to the subjects following the dose in each period. It will be the same in quantity and content as that provided in the previous period.

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

12.5. Drugs Classification According to their Risk for Pregnancy and Breastfeeding (FDA)

A	**Without apparent risks**	Controlled studies in women do not show risk for the fetus during the first quarter and the likelihood of fetal injuries seems remote. ***They can be used.***
B	**With apparent risks**	Animal studies do not show risk for the fetus, and there are no controlled studies in humans, or the animal studies do show an adverse effect for the fetus, but in well controlled studies in pregnant women they have not showed fetal risk. ***Probably safe.***
C	**Undetectable risk**	Animal studies have shown that the drug has teratogenic or embryocidal effects, but there are not controlled studies in women, or there are not studies in animals or women. ***Avoid them if there is any other choice.***
D	**Proven risk**	There is positive evidence of fetal risks in humans, but, in some cases (e.g., in life-threatening situations or in serious diseases where safer drugs cannot be used, or those which can be used are inefficient), benefits may turn the drug acceptable despite its risks. ***Avoid them if there is any other choice.***
X	**Contraindicated**	Studies in animals or in human beings have shown fetal abnormalities; or there is fetal risk based on the experience in human beings; or are applicable to both cases. Risks clearly overcome the possible benefit. ***Contraindicated.***