Statistical Analysis Plan

Study ID: 212969

Official Title of Study: An oral single-dose, randomized, balanced, open-label, two-sequence, two-treatment, two-period, crossover bioequivalence study of Paroxetine tablets 20 mg of GlaxoSmithKline Pharmaceuticals S.A, with that of P AXIL (Paroxetine) tablets 20 mg of GlaxoSmithKline Mexico S.A. de C.V., in healthy adult male and female subjects under fasting conditions.

Date of Document: 21-JAN-2021

TITLE

An oral single-dose, randomized, balanced, open-label, two-sequence, two-treatment, two-period, crossover bioequivalence study of Paroxetine tablets 20 mg of GlaxoSmithKline Pharmaceuticals S.A, with that of PAXIL (Paroxetine) tablets 20 mg of GlaxoSmithKline México S.A. de C.V., in healthy adult male and female subjects under fasting conditions.

PROTOCOL NO: **AS/BK/OCT-19/0055**

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Statistical Analysis Plan of Bioequivalence Pivotal Study of Paroxetine tablets 20 mg under fasting conditions.

1.0 Introduction

Paroxetine is an antidepressants drug. It is the hydrochloride salt of a phenylpiperidine compound identified chemically as (-)-trans-4R-(4'fluorophenyl)-3S-[(3',4'-methylenedioxyphenoxy) methyl] piperidine hydrochloride hemihydrate and has the empirical formula of $C_{19}H_{20}FNO_3 \cdot HCl \cdot 1/2H_2O$.

Paroxetine is indicated for the treatment of:

- Major depressive disorder.
- Obsessive Compulsive Disorder (OCD).
- Panic disorder with and without agoraphobia.
- Social anxiety disorders/social phobia.
- Generalised anxiety disorder.
- Post-traumatic stress disorder.

2.0 Study Objective

Primary Objective

To evaluate and compare the single oral dose bioavailability of Paroxetine tablets 20 mg manufactured by GlaxoSmithKline Pharmaceuticals S.A. for GlaxoSmithKline México, S.A. de C.V. with that of PAXIL (Paroxetine) tablets 20 mg of GlaxoSmithKline México, S.A. de C.V. in healthy, adult, male and female subjects under fasting conditions.

Secondary Objective

To evaluate the safety and tolerability of single oral dose of Paroxetine 20 mg tablets in healthy adult male and female subjects under fasting conditions.

3.0 Hypothesis of the study

Null hypothesis: H_0: mean test / mean reference < L or mean test / mean reference > U

Paroxetine tablets 20 mg manufactured by GlaxoSmithKline Pharmaceuticals S.A. and PAXIL (Paroxetine) tablets 20 mg of GlaxoSmithKline México, S.A de C.V. are not bioequivalent.

Alternative hypothesis: H_1: L ≤ mean test / mean reference ≤ U

Paroxetine tablets 20 mg manufactured by GlaxoSmithKline Pharmaceuticals S.A. and PAXIL (Paroxetine) tablets 20 mg of GlaxoSmithKline México, S.A. de C.V. are bioequivalent.

Two one-sided t-tests will be carried out on each PK parameter of Paroxetine using least squares means (LSM) values for geometric LSMs of the primary PK parameters (mean test or mean reference = LSM value of the corresponding PK parameter for test or reference product). The type I error will be set to $\alpha = 5\%$ and therefore 90% (two-tailed) confidence intervals will be provided together with the indication whether the null hypothesis of nonequivalence for the appropriate parameter can be rejected.

Probabilities p(t) of Schuirmann, left tail (t_l) and right tail (t_r) values will be calculated for the lower limit L = 0.80 and upper limit +U = 1 / 0.80 = 1.25 for geometric LSM of pharmacokinetic parameters C_{max} and AUC_{0-t}.

4.0 Study design

An oral, single-dose, randomized, balanced, open-label, two-sequence, two-treatment two-period, crossover comparative bioavailability study under fasting conditions.

The study design is presented in Figure 1 below.

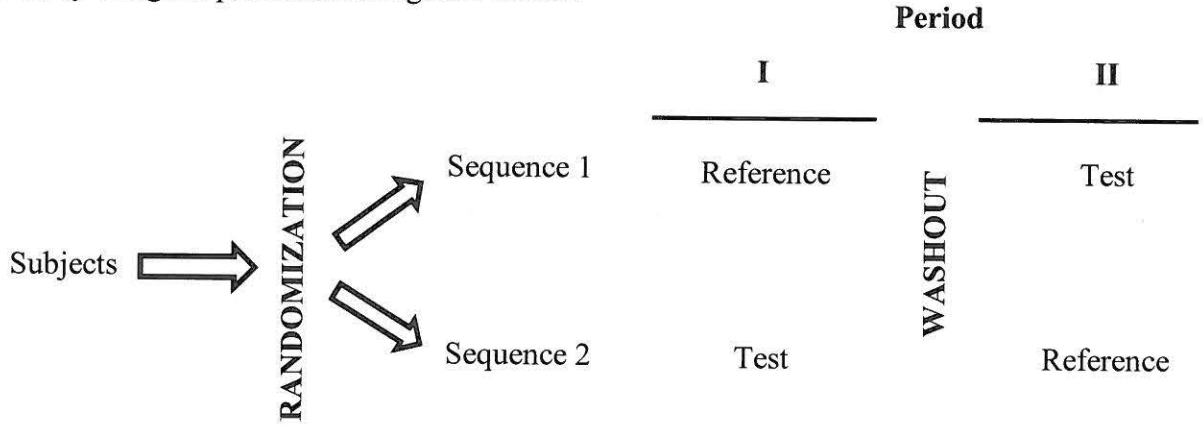

Figure 1. Study Design Diagram

5.0 Randomization

The order of receiving the test and reference products for each subject during each period of the study will be determined according to a randomization, generated by using SAS® software (version 9.4 or higher). This randomization will be balanced for the test product (A) and reference product (B). All the subjects should be divided into blocks of equal size and two-sequence "AB" and "BA" will be used for assignment of treatment in the respective study period (refer Figure-1). Analysts will not have access to the period randomization schedule.

Randomization will be performed according to the procedure PB009 (Procedure for Randomization Schedule Generation).

Blinding

This study is an open label study; the subjects and the investigators will not be blinded towards the identity of the study treatments. Analysts (bioanalytical personnel) will be blinded with regard to the sequence of administration of test and reference products.

6.0 Sample size

At least 34 healthy adult male and female subjects are required to meet at least a statistical power of 90% at 5% level of significance to achieve the bioequivalence results within 80.00-125.00% in this study. In addition, a maximum of 4 subjects will be dosed in account for withdrawal and dropouts.

Hence, a maximum of 38 subjects will be randomized and dosed. No subjects should be replaced after administered with the study treatment.

If possible, a maximum of two additional standby subjects (Stand by-I, Stand by-II) will be enrolled to replace any subject, who is withdrawn or dropped due to any reason prior to administration of study medication in period-1.

7.0 Justification of the Sample Size

Based on the in-house study data [11], a maximum intra subject variability of coefficient of variation (ISCV %) of 23.1 was observed for C_{max} of Paroxetine. According to NOM-177-SSA1-2013, section 8.5.1.1, the statistical power must not be less than 80%. Based on the observed maximum ISCV% of 23.1% and assuming the test/reference ratio of 95%, the inclusion of 34 subjects in the study is required to meet at least a statistical power of 80% at 5% level of significance to achieve the results within 80.00-125.00% for 2-way crossover study.

Using the intra-subject variability and the expected test/reference ratio from above paragraph, the sample size is estimated from the following equation:

$$n_i \geq 2\,[t_{1-\alpha,\,n-2}+t_{1-\beta,\,n-2}]^2[CV/(-\ln(\theta_L)-(-\ln(\theta))]^2 \text{ where } \theta \leq 1$$

α: Probability of Type I error (0.05)

β: Probability of Type II error (0.1)

CV: Intra Subject Variability (0.231)

θ: Expected T/R ratio (0.95)

θL: Lower bioequivalence limit (0.80)

Assuming the minimum sample size required (n) as 12, the sample size is estimated as follows:

$$n_i \geq 2\,[t_{1-0.05,\,12-2}+t_{1-0.1,\,12-2}]^2\,[0.231/(-\ln(0.8)-(-\ln(0.95))]^2$$

$$n_i \geq 37$$

Using the estimated sample size form previous iteration (n=37) from the above estimation, the sample size is again estimated as follows:

$$n_i \geq 2\,[t_{1-0.05,\,37-2}+t_{1-0.1,\,37-2}]^2\,[0.231/(-\ln(0.8)-(-\ln(0.95))]^2$$

$$n_i \geq 32$$

Using the estimated sample size form previous iteration (n=32) from the above estimation, the sample size is again estimated as follows:

$$n_i \geq 2\ [t_{1-0.05,\ 32-2}+t_{1-0.1,\ 32-2}]^2\ [0.231/\ (-\ln\ (0.8) - (-\ln\ (0.95))]^2$$

$$ni \geq 33$$

Using the estimated sample size form previous iteration (n=33) from the above estimation, the sample size is again estimated as follows:

$$n_i \geq 2\ [t_{1-0.05,\ 33-2}+t_{1-0.1,\ 33-2}]^2\ [0.231/\ (-\ln\ (0.8) - (-\ln\ (0.95))]^2$$

$$n_i \geq 33$$

Convergence has reached and the estimated sample size is at least 34 (round to equal number) subjects to meet at least a statistical power of 80% at 5% level of significance to achieve the results within 80.00-125.00% for 2-way crossover study.

8.0 Washout period

There will be a washout period of at least 7 days between successive dosing.

9.0 Duration of the study

The expected duration of the subjects' participation in the study will be at-least 12 days, including a wash-out period of 7 days between each dosing.

10.0 Test and Reference Products

Table 1. Investigational Product Details

IP Details	Reference Product (B)	Test Product (A)
Distinctive Name	PAXIL	N/A
Generic name	Paroxetine [Paroxetine as Paroxetine hydrochloride hemihydrate]	Paroxetine [Paroxetine as Paroxetine hydrochloride hemihydrate]
Pharmaceutical form	Tablets	Tablets
Formulation	Immediate release	Immediate release
Strength	20 mg	20 mg
Dose	20 mg	20 mg
Lot / Batch No.	1930200023	YC4M
Expiry date / Re-test date	Oct/2021	28/Nov/2021
Storage Conditions	Store at 25°C (77°F): excursions permitted to 15–30°C (59–86°F).	Store at 25°C (77°F): excursions permitted to 15–30°C (59–86°F).
Name of the manufacturer	GlaxoSmithKline México, S.A. de C.V.	Manufactured by: GlaxoSmithKline Pharmaceuticals S.A. Manufactured for: GlaxoSmithKline México, S.A. de C.V.
Sanitary Registration Number	008M93SSA	Not applicable [1]

1: The test drug will be evaluated for obtaining COFEPRIS registration.

11.0 Sampling Schedule

In each period, a total of 24 blood samples (4 mL each) will be collected from each subject as per the following schedule:

- The first blood sample will be collected within 1.50 hours prior to the dosing (00.00 hours / pre-dose).
- The remaining samples will be collected after the dosing of the test or reference drug at 00.50, 01.00, 01.50, 02.00, 02.50, 03.00, 03.50, 04.00, 04.50, 05.00, 05.50, 06.00, 06.50, 07.00, 08.00, 09.00, 10.00, 12.00, 16.00, 24.00, 36.00, 48.00 and 72.00 hours.
- The samples corresponding to 36.00, 48.00 and 72.00 hours post dose will be collected on separate visits as ambulatory sample in each period of the study.
- Blood samples shall be collected not earlier or later than (±) 2 minutes of the specified sampling time for all the in-house samples and not earlier or later than (±) 60 minutes of the specified sampling time for ambulatory samples. Blood sampling will be registered in the respective source documents.
- If blood samples are collected earlier or later than this period from the scheduled time, this shall be reported as a deviation from this protocol.
- If any subject misses any blood sampling point, it will be considered as missing sample.
- The same will be denoted as (M), it will be considered as protocol deviation and mentioned in the clinical study report.

12.0 Assessment of efficacy

As this is a bioequivalence study, no efficacy assessment will be performed. The pharmacokinetic parameters of the test and reference formulations will be assessed.

13.0 Pharmacokinetic parameters

Non-compartmental pharmacokinetic analysis will be performed on the observed plasma concentration of Paroxetine using WinNonlin® software (6.4 version or higher).

- Any concentration below lower limit of quantification (BLOQ) including pre-dose concentrations will be converted to zero.
- If the pre-dose concentration is ≤ 5% C_{max} of the respective period and above LLOQ, then pre-dose concentration will not be converted to zero and the respective subject shall be considered for pharmacokinetic and statistical analysis.

Missing samples will be reported as "NE" and will not be included for pharmacokinetic and statistical analysis. The process will be performed according to the procedure PB003 (Procedure for Statistical Analysis using WinNonlin).

The following pharmacokinetic parameters will be computed.

Primary parameters

C_{max}	:	Maximum observed concentration following each treatment.
AUC_{0-t}	:	The area under the concentration versus time curve from time zero to the last measurable concentration using linear trapezoidal linear interpolation method.

Secondary parameters

T_{max}	:	Time of the maximum measured concentration.
AUC_{0-inf}	:	The area under the concentration versus time curve from time zero to infinity calculated; Where $AUC_{0-inf} = AUC_{0-t} + C_t/ \lambda z$, Ct is the last measurable concentration and λz is the terminal elimination rate constant.
AUC_% Extrap	:	The residual area in percentage $[(AUC_{0-inf} - AUC_{0-t})/ AUC_{0-inf}] \times 100$
Lambda_z (K_{el})	:	First order rate constant associated with the terminal portion of the curve (log-lineal). This is estimated via linear regression of

time vs. log concentration. This parameter will be calculated using at least three or more non-zero plasma concentration values.

HL_Lambda_z ($t_{½}$) : The elimination half-life will be calculated as 0.693/ λz

Note:

- For all the above computations, actual time points of the sample collection will be used in case of sample collection deviations i.e. beyond ± 2 minutes.
- No value of Lambda_z (K_{el}), $AUC_{0\text{-}inf}$, or HL_Lambda_z ($t_{½}$) will be reported for cases who do not exhibit a terminal Log-linear phase in the concentration versus time profile.
- Statistical methods used in the study will be mentioned in the following section.

14.0 Statistical analysis

Data from all treated subjects will be included in the statistical analysis.

If any of the subject's data is eliminated from the statistical analysis which meet the criteria of section 14.9 'Criteria for elimination of subject data from the statistical analysis', statistical analysis shall be performed and presented with and without the data of eliminated subject's data.

The statistical analysis will be carried out according to the PB005 procedure (Procedure for Statistical Analysis for a cross-over design).

Statistical analysis of PK parameters for establishing bioequivalence will be performed using the software SAS® (version 9.4 or higher). PROC GLM will be used for the estimation of the geometric least square mean differences (test - reference) of the test and reference formulations on the ln-transformed PK parameters C_{max} and $AUC_{0\text{-}t}$ for Paroxetine and the corresponding standard errors of the differences will also be computed. Based on these parameters, 90% confidence intervals will be constructed for

the geometric least square mean differences of ln-transformed C_{max} and AUC_{0-t} for Paroxetine.

The anti-ln (or exponential) of the limits obtained from the ln-transformed data will give the 90% confidence interval for the ratio of geometric least square means of test and reference formulations.

14.1 Descriptive Statistics

Geometric mean, arithmetic mean, median, minimum, maximum, standard deviation and coefficient of variation (arithmetic and geometric, respectively) will be calculated for each PK parameter (C_{max}, AUC_{0-t} and T_{max} for Paroxetine), logarithmically transformed and untransformed.

For PK parameters C_{max} and AUC_{0-t} for Paroxetine, the following shall be calculated:

- Difference of test and reference drugs.
- Ratio between test and reference drugs.
- The natural logarithm of the Ratio between test and reference drugs.
- Histograms (ratios [A/B] and ln ratio [Ln (A/B)] will be performed.

14.2 Analysis of Variance

The ln-transformed pharmacokinetic parameters (C_{max}, AUC_{0-t} for Paroxetine) will be analyzed using Type III sum of squares, with the main effects of formulation, period, sequence as fixed effects and subjects nested within sequence as a random effect. A separate ANOVA model will be used to analyze each of the parameters. The sequence effect will be tested at the 5% level of significance using the subjects nested within sequence mean square as the error term. Formulation and period effects will be tested at the 5% level of significance against the residual error (mean square error) from the ANOVA model as the error term. Each analysis of variance will include calculation of geometric least-squares means, the difference between the adjusted formulation means and the standard error associated with the difference.

14.3 Statistical Model

The statistical model used in this research is a general linear model, whose equation is:

$$Y_{ijkl} = \mu + F_i + Seq_j + P_k + S_l(Seq_j) + e_{ijkl}$$

Where:

Y_{ijkl} = Response of l^{th} subject of j^{th} Sequence when it received i^{th} Formulation in k^{th} Period

μ = general mean

F_i = fixed effect of i^{th} formulation

Seq_j = fixed effect of j^{th} sequence

P_k = fixed effect of k^{th} period

$S_l(Seq_j)$= random effect of l^{th} subject nested within the j^{th} sequence

e_{ijkl} = random error

In addition, as the study was conducted in groups, group effect will be tested for informative purpose using the below mentioned model.

$$Y_{ijklm} = \mu + F_i + Seq_j + P_k + Gm + S_l(Seq_j \times G_m) + F_i \times G_m + e_{ijklm}$$

Where:

Y_{ijklm} = Response of l^{th} subject of j^{th} Sequence when it received i^{th} Formulation in k^{th} Period for m^{th} group

μ = general mean

F_i = fixed effect of i^{th} formulation

Seq_j = fixed effect of j^{th} sequence

P_k = fixed effect of k^{th} period

Gm = fixed effect of m^{th} group

$S_l(Seq_j \times G_m)$ = random effect of l^{th} subject nested within the j^{th} sequence for m^{th} group

$F_i \times G_m$= effect of i^{th} formulation for m^{th} group

e_{ijkl} = random error

14.4 Two One-Sided Test for Bioequivalence

A two one-sided test (Schuirmann) for the differences of least squares means between the formulations (test/reference) will be calculated for ln-transformed data of the C_{max} and AUC_{0-t} for Paroxetine.

The hypothesis approach for the Schuirmann two one-sided test is:

Null hypothesis H_0: $\mu_{test} / \mu_{ref} < (0.8)$ H_{0a}: $\mu_{test} / \mu_{ref} > (1.25)$ (not bioequivalent)

Alternative hypothesis H_1: $\mu_{test} / \mu_{ref} \geq (0.8)$ H_{1a}: $\mu_{test} / \mu_{ref} \leq (1.25)$ (bioequivalent)

Acceptance criteria: to support bioequivalence, the null hypothesis in both conditions shall be rejected with a significance level of 5% for each side. With regards to the classical generated 90% confidence interval, it shall fall within the bioequivalence range of 80.00% - 125.00%.

14.5 Statistical Power

Statistical power is the probability of rejecting the null hypothesis when it is false. Power is 1-Type II (where Type II error is the false negative error). The statistical power will be reported but considered only as informative when the test product is bioequivalent to the reference product.

14.6 Ratio Analysis

Ratio of geometric least squares means of the test and reference drugs will be computed for un-transformed and ln-transformed primary pharmacokinetic parameters C_{max} and AUC_{0-t} for Paroxetine.

14.7 Intra-Subject Variability

Intra-subject variability will be computed for un-transformed data and for ln-transformed Pharmacokinetic parameters C_{max} and AUC_{0-t} for Paroxetine.

14.8 Analysis of Outliers

The outliers shall be identified (< -2.00 and > +2.00) using a ±2 studentized residuals using the software SAS® version 9.4 or higher and their exclusion in the statistical analysis shall be justified (if applicable).

The subject's data will not be excluded from the statistical analysis exclusively due to mathematical or statistical reasons.

If statistical analysis concluded after removing the outliers, the results shall be submitted with and without the inclusion of outliers with a justification. In this case, the persons responsible for the study, along with quality assurance personnel will investigate the possible causes of the appearance of outliers, and scientific evidence will be provided in addition to the statistical evidence.

14.9 Criteria for elimination of subject data from the statistical analysis.

No data shall be eliminated from statistical analysis, except for the following.

a. Research (study) subjects with pre-dose concentrations in plasma:

   If the pre-dose concentration is 5% of C_{max} in that research subject, the data can be included without adjustments in all pharmacokinetic measurements and calculations. In cases where the pre-dose sample is >5% of the C_{max}, the research subject shall be eliminated from all bioequivalence assessments.

b. Data elimination due to vomiting or diarrhea.

   Since the study drug is an immediate release product; the data from research subjects who experience vomiting or diarrhea occur before 2 times the median T_{max} or 2 times the value of T_{max} obtained in the research subject in a given period shall be eliminated from all bioequivalence assessments.

c. A research subject who lacks measurable concentrations or with plasma concentrations well below that of the reference drug.

   A research subject is considered to have very low concentrations, if the AUC is less than 5% of the AUC geometric mean of the reference drug (shall be calculated without inclusion of the atypical values of the research subject data). The exclusion of data due to this reason will be accepted only with a scientific justification and with prior review by COFEPRIS.

d. Since the planned study is a crossover and balanced, subjects who did not complete in any of the study periods (subject that is discontinued or withdrawn) shall be eliminated from all bioequivalence assessments.

The AUC_{0-t} shall cover at least 80% of the AUC_{0-inf}. The research subjects shall not be excluded for the statistical analysis if the AUC_{0-t} covers less than 80% of the AUC_{0-inf}.

In case that a research subject elimination proceeds, according to the above-mentioned information, the excluded research subject samples shall be analyzed and the results shall be presented. Also, the statistical analysis shall be performed and shall be presented with and without the data of eliminated research subjects.

14.10 Bioequivalence Criteria

Based on the statistical results of classic 90% confidence interval for the ratio of the geometric least squares means for log-transformed PK parameters C_{max} and AUC_{0-t} for Paroxetine, a conclusion will be drawn for test product (A) vs. the reference product (B) under fasting conditions.

The bioequivalence of the test product (A) with that of the reference product (B) under fasting conditions will be concluded if the 90% confidence interval of the ratio of the test and reference product (test/reference) falls within the acceptance range of 80.00 – 125.00% for ln-transformed PK parameters C_{max} and AUC_{0-t} of Paroxetine.

15.0 Amendments and Protocol Deviations

A protocol deviation is an unintended excursion from the approved protocol.

A major protocol deviation is a protocol deviation that may, as evaluated by the principal investigator, or person designated by the investigator impact the safety of the subject or the integrity of the trial. Major deviations will lead to withdrawal of a subject from the study. Deviations not classified as major are considered as minor protocol deviations.

All protocol deviations should be reported to the sponsor by the Avant Santé and monitor (if present on site) at the earliest possible time. The sponsor can propose to re-classify a protocol deviation (minor to major or vice versa) upon evaluation. In such case, the classification made by the sponsor prevails and will be communicated to Avant Santé together with a written justification.

Major protocol deviations will only be implemented after sponsor's approval and obtaining approval or a favorable opinion from the Research Ethics Committee (REC) and Research Committee (RC) and COFEPRIS.

The sponsor must be informed of minor protocol deviations, but before start of the following clinical study period or before start of the bioanalytical phase/ statistical phase.

Minor protocol deviations will be reported sponsor and Committees if applicable, and will be reported in study report:

- Logistic deviations (follow up visits that occurred outside the protocol required time frame because of the participant's schedule etc.)
- All post dose in-house blood samples collected beyond ± 2 minutes during housing period and ± 60 minutes for ambulatory samples from the scheduled collection time will be reported as protocol deviations.
- Deviations related to meal consumption.
- Administrative deviations (e.g. change of names).

Protocol deviation notification and reports are submitted to Health Authorities and/or relevant Research Ethics Committee (REC) and Research Committee (RC) according to applicable requirements/guidelines/law.

Procedure for documenting protocol deviations

The Principal Investigator, or person designated by PI, should document and explain any deviation from the approved protocol. The notification of protocol deviation to sponsor can be in exceptional cases communicated by verbal means (if immediate

action/notification is needed) and must be followed-up with written documentation (e.g. by e-mail; period update). All major protocol deviations, as well as time point deviations or meal plan, must be described in the final study report.

16.0 References

1. Protocol of the study AS/BK/OCT-19/0055 Version 01
2. Protocol of the study AS/BK/OCT-19/0055 Version 02
3. NORMA Oficial Mexicana NOM-177-SSA1-2013, Que establece las pruebas y procedimientos para demostrar que un medicamento es intercambiable. Requisitos a que deben sujetarse los Terceros Autorizados que realicen las pruebas de intercambiabilidad. Requisitos para realizar los estudios de biocomparabilidad. Requisitos a que deben sujetarse los Terceros Autorizados, Centros de Investigación o Instituciones Hospitalarias que realicen las pruebas de biocomparabilidad, Sep, 2013.
4. Guideline for Good Clinical Practice, E6 (R2), International Council on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use, Current Step 5 version, 15 December 2016.
5. Food and Drug Administration (FDA), Guidance for Industry: Statistical approaches to establishing bioequivalence 2001.
6. Schuirman, D. J. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. J. Pharmacokin. Biopharm., 1987, 715:657-680.
7. Hauck, W.W., Anderson, S. A New Statistical Procedures for testing equivalence in two group comparative bioavailability trials. J. Pharmacokin. Biopharm., 1984, 12:83-91.
8. SOP PB009, Generation of Randomization Schedule).
9. SOP PB003, Statistical Analysis for Bioequivalence Studies Using Winnonlin® Software.
10. SOP PB005, Procedure for Statistical Analysis of Crossover Design.

11. "INFORME FARMACOCINÉTICO – ESTADÍSTICO" of In-house study-AS/AO/ABR-17/0012 (Paroxetine 20 mg).

17.0 Change history

Current Version No.	Supercedes (Version No.)	Changes Made
00	N/A	N/A
01	00, 26/Nov/2020	1. Section 14.3: The statistical model is included to evaluate the Group Effect by means of ANOVA, after conducting the study in groups.