Bioequivalence Study of Two Formulations of Escitalopram Oxalate 20 mg Tablets in Healthy Volunteers

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Abstract

A Bioequivalence study was developed to compare the bioavailability of two formulations of Escitalopram oxalate 20 mg tablets in twenty-four healthy volunteers. The Test product was Escitalopram made by Laboratorios Tecnoquímicas S.A. (Jamundí – Colombia) and the Reference product was Lexapro® (Escitalopram) made by H. Lundbeck A/S (Valby - Denmark). A crossover, 2 x 2, single-dose, two treatments, two periods, two sequences design was used, with a washout period of one week. Prior to dose administration a basal blood sample was taken and between 1 and 96 hours after the administration another 17 samples were collected. The determination of Escitalopram in plasma was performed using a bioanalytical method for ultra high resolution liquid chromatography coupled to a single quadrupole mass spectrometer (UHPLC-MS) and using ESI+, previously validated. Through Escitalopram concentration curves versus time obtained from measurements in the plasma of volunteers the pharmacokinetic parameters and Bioequivalence were determined for both products. The pharmacokinetic parameters determined in this study to the Reference and Test products were C_{max} 15.7 ± 7.3 ng/ml, 14.5 ± 5.9 ng/ml, $AUC_{0\to\infty}$ 901.6 ± 389.2 ng.h/ml, 731.3 ± 257.1 ng.h/ml and $AUC_{0\to96}$ 740.9 ± 354.0 ng.h/.ml, 612.7 ± 207.6 ng.h/ml respectively. For Escitalopram, the ratio of the logarithmic transformation Test product / Reference product for $AUC_{0\to96}$ were from 94.6 to 103.1 and for C_{max} were from 91.7 to 107.4; both ratios with confidence interval of 90%. These intervals are within the range of Bioequivalence established and therefore it can be concluded that the Test formulation is interchangeable or Bioequivalent to the Reference.
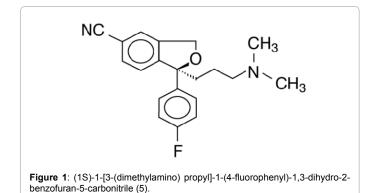
Keywords: Escitalopram; Bioequivalence; Pharmacokinetics; Ultra high resolution liquid chromatography; Mass spectrometry

[*]The test product is manufactured by Laboratorios Tecnoquímicas S.A. in Jamundí, Colombia and commercialized in Colombia and Ecuador as Escitalopram[*] MK and in Central America as Escitaloteg[*] TG.

Introduction

Escitalopram, $C_{20}H_{21}FN_{2O}$ [1,2] (Figure 1) is the S enantiomer of citalopram. It is used to treat depression, generalized anxiety disorder, worry and tension. It belongs to a class of antidepressants called selective serotonin reuptake inhibitors and it acts by increasing serotonin concentrations, which is a natural substance in the brain that helps to maintain mental balance [3].

Maximum plasma levels are reached about 3 ± 1.5 hours after a single dose of an Escitalopram 20 mg tablet [4]. This active ingredient binds to human plasma proteins in approximately 56% and it is mainly metabolized in the liver to less lipophilic compounds; demethyl-escitalopram and didemethyl-escitalopram [4,5]. The primary route of excretion of Escitalopram and its metabolites is via the kidneys, with a little percentage excreted in the feces. The elimination half-life is about 30 hours [5,6].

The aim of this study was to establish the Bioequivalence of two formulations of Escitalopram oxalate, comparing the Bioavailability of a single dose of Escitalopram 20 mg tablets made by Laboratorios Tecnoquímicas S.A. (Test product) against a single dose of Lexapro[*] 20 mg tablets made by H. Lundbeck A/S (Reference product).

Study of the Products

The batches 3K2642A, 4A0529 and 3P3479B from the Test product Escitalopram 20 mg coated tablets were used. These were manufactured by Laboratorios Tecnoquímicas S.A (Jamundí - Colombia). The batch 2356043 from the Reference product Lexapro[*] 20 mg coated tablets was used, manufactured by H. Lundbeck A/S (Valby - Denmark).

Prior to the Bioequivalence study development, the products were compared in presentations, label, prospects and tablets description. Also, identity and content of active ingredient, uniformity of dosage units, dissolution test and profiles in three different media (HCl 0.1 M pH=1.2, $NaH_2PO_4.H_2O$ 50 mM pH=4.5, Na_2HPO_4 50 mM pH=6.8) were evaluated by analytic methods, following a monograph from the

Figure 1: (1S)-1-[3-(dimethylamino) propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-carbonitrile (5).

United States Pharmacopoeia USP 36 NF 31 and the FDA guideline [7,8]. This was performed in order to determine the pharmaceutical equivalence between the Test and Reference drug.

Products are accepted as pharmaceutical equivalents and appropriated to the Bioequivalence study if: in the analysis of active ingredient identity the products Escitalopram peak retention time matches with the standard; in the study of content of active ingredient and uniformity of dosage units the products have between 90.0 and 110.0% of the declared quantity; in the dissolution test the dissolved percentage in 30 minutes is not less than 80% and if in dissolution profiles the values of f_1 "point to point differences" are between 0 and 15, and of f_2 "similarity test" are between 50 and 100, and the coefficient of variation for dissolved Escitalopram in the first sample time is 20% maximum and less than 10% for the other times [7,8].

Subjects and Methods

Subjects

Volunteers were enrolled by open call performed by the Centro de la Ciencia y la Investigación Farmacéutica (CECIF), site where the study was developed and has experience in drug analysis since the year 1997; this site is located in Medellín- Colombia. The call took place in different universities of the area, on line by the web site: www.cecifcolombia.org and by informative talks about the study.

Volunteers were healthy Colombian of both sexes, between 20 and 28 years old. With a 15% limit weights difference according to the height [9] and a BMI between 18.1 and 25.1 Kg/m^2.

The following variables were taken into account to evaluate the inclusion of subjects in the study: no history of liver, heart, kidney, blood, central nervous system or respiratory disease; besides normal blood pressure (in adults the systolic pressure must be less than 120–130 mmHg and the diastolic pressure must be 80–90 mmHg) and appropriate heart rate (the normal heart rate at rest is from 60 to 100 beats per minute). Also, normal results of performed clinical tests (Complete blood count, Fasting blood glucose, Analysis of amino-transferase, total cholesterol, serum creatinine, serum triglycerides, HIV test, serum albumin, urinalysis) and negative pregnancy test.

All volunteers were informed about the nature of the study and a written informed consent was obtained from each one of them. They were asked not to take any drugs, enzymatic inducer or even contraceptives for at least two weeks before the first period of sampling [10,11], to avoid taking food or drinks that contain xantines: chocolate, tea, coffee and cola drinks, not to drink alcoholic products and cigarettes 48 hours before dose administration. Volunteers had 10 fasting hours before the administration of Escitalopram 20 mg tablet and could have breakfast only 2.5 hours after the dose administration.

Study design

The protocol and informed consent form for the present study were approved by the Bioethics Committee of the Sede Investigación Universitaria de la Universidad de Antioquia CBE-SIU, Medellin, Colombia, which is governed by resolution 008430 of October 4, 1993 of the Ministry of Health of Colombia [12], which states the technical scientific and administrative rules for health research and follows the guidelines of resolution 002378 June 25, 2008 of the Ministry of Social Protection [13], which sates the Good Clinical Practice for institutions that conduct research with drugs in human beings. Besides, it corresponds to the principles of the World Medical Assembly exposed in the Declaration of Helsinki of 1964, last revision in 2008 [14] and

the Code of Federal Regulations, Title 45, Part 46, for the protection of human subjects, of the Department of Health and Human Services of National Institutes of Health in the United States (June 18, 1991) [15].

For the present study, a crossover, 2x2, randomized, single dose, with two treatments, two periods and two sequences design was used, with a wash out period of 9 days. Each subject randomly received a treatment sequence and all of them received the Escitalopram Test and Reference products.

Drug administration

Volunteers were gathered for more than 12 hours in an area of the Corporación para estudios en salud, CES clinic (Medellin-Colombia) to collect the samples. All of them underwent the same housing conditions, such as food intake, drink volume intake, physical activity and position when lying, among others. One hour before dose administration, an intravenous catheter was placed to each volunteer, and a blank blood sample was taken. The dose was orally administered with 240 mL of water.

Sampling

Blood samples were drawn from the ante-cubital vein of each volunteer, in tubes with anticoagulant (sodium heparin), at the following times: 0, 1, 2, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 8, 10, 12, 24, 48 and 96 hours. In an approximate range of time of 20 minutes after taking the samples, tubes were centrifuged at 3500 rpm to obtain the blood plasma; this was stored in sterile tubes and immediately put at -20ºC until the time of analysis.

During both periods of study, volunteers were under medical care with a permanent monitoring of vital signs to prevent or counteract any adverse reaction.

Analysis of plasma samples

Escitalopram concentrations in plasma were measured using a bioanalytical method for ultra-high performance liquid chromatography coupled to a single quadrupole mass spectrometer (UHPLC-MS) and in ESI+ mode, previously validated by CECIF following the parameters established in the international guidelines of analytical methods of validation [16-21], to demonstrate an adequate sensibility, specificity, linearity, accuracy and precision.

Conditions for Escitalopram extraction from plasma and chromatographic conditions for its quantification were determined after a previous revision of scientific literature, implementing necessary modifications to its optimization [22-26].

Sample preparation was made as follows: To 500 μL of tempered plasma, 500 μL of acetonitrile are added and the mixture is then vortexed (2000 RPM) for 3 minutes, subsequently it is taken to centrifugation for 15 minutes at 13,180 RPM (20,000 RCF) and 0ºC. The supernatant is removed and brought back to the centrifugal continuing with the same parameters described above. The supernatant is pressure filtered using 0.22 micron Nylon filters in 2mL vials, and then injected into an ultra-high performance liquid chromatography coupled to a single quadrupole mass spectrometer (MS). A Shimadzu UFLC-NEXERA chromatograph equipment with a liquid chromatography detector LC-MS 2020 was used. Data was obtained using the LabSolutions 5.53 SP3 Shimadzu Corporation software. Separation of analyte was performed with a Zorbax Eclipse XDB-C18 2.1 × 5 mm, 1.8 μm guard column, coupled to an Agilent, RRHD Extend - C18, 2.1 × 50 mm, 1.8 μm chromatographic column at 1200 bar and 35ºC temperature. Isocratic

elution was performed with an acetonitrile mobile phase: Ammonium acetate Buffer Solution 2.0 mM pH: 5.00, 45:55, at a constant flow of 0.3 mL/min. The total runtime was 3.0 min. Escitalopram was detected with an ESI+ ionization mode and monitoring the 325 ion. The injection volume in the UHPLC was 2.0 μL.

Pharmacokinetic parameters analysis

Levels of Escitalopram for each volunteer at each time were tabulated; from these data the concentration curves of Escitalopram in plasma vs. time for both formulations were made. The pharmacokinetic parameters compared in this single dose Bioequivalence study for both formulations are the following: C_{max} and t_{max} that correspond to the curve peak, $AUC_{0 \rightarrow 96}$ calculated by the trapezium method, area under the curve from the last sample time t to infinite ($AUC_{96 \rightarrow \infty}$) determined by the $AUC_{t \rightarrow \infty} = C_{96}/K_e$ equation, $AUC_{0 \rightarrow \infty}$ thus, $AUC_{0 \rightarrow \infty} = AUC_{0 \rightarrow 96} + AUC_{96 \rightarrow \infty}$, both K_e and $t_{1/2}$ were found from the slope of the end elimination phase of the curve.

The statistic method used to determine the Bioequivalence between formulations was based on a two one-sided test [27], to determine whether the mean transformed values of pharmacokinetic parameters measured after the Test and Reference products administration were comparable.

For the statistical analysis of the data derived from this *in vivo* study, general parametric procedures of linear models (normal theory) were used, and an analysis of variance (ANOVA) was made using the Phoenix^{TM} Winnonlin$^\circ$ 2013 and Excel 2014 statistical packages from the $LogC_{max}$ and $logAUC_{0 \rightarrow \infty}$ pharmacokinetic parameters, taking into account the mean concentration profiles of volunteers grouped in categories, also some potential variation sources were included such as: sampling periods and individual area calculation for each treatment, as the FDA Bioequivalence Division establishes [28]. It can be concluded that the formulations are Bioequivalent if the relation of $LogAUC_{0 \rightarrow 96}$ and $LogC_{max}$ between the Test and Reference products for a 90% confidence interval is within the ranges of Bioequivalence of 80 to 125 established by the FDA [28-31].

Results

Study of the products

The presentations, labels, prospects and tablets comparison, according to the Pharmacopoeia (USP 36), did not show relevant differences between both products. The active ingredient identity analysis showed the same Escitalopram retention time for the standard in mobile phase and the four samples prepared from the Reference drug batch and the three Test drug batches, demonstrating the Escitalopram identity. The analytical results of the active ingredient, the uniformity of dosage units and the dissolution test, are summarized in Tables 1 and 2, showing the accomplishment of specifications [7,8]. As to the

Product	Medium					
	pH 1,2		pH 4,5		pH 6,8	
	f_1	f_2	f_1	f_2	f_1	f_2
Test 3K2642A	0.77	89.21	0.83	88.04	1.01	98.85
Test 4A0529	0.52	89.29	2.17	65.84	0.52	90.77
Test 3P3479B	0.44	92.50	1.17	75.59	0.75	85.67
Specification	Limits must be: f_1: 0 – 15, f_2: 50 - 100					
Result	Comply					

Table 1: Results of in-vitro bioequivalence for dissolution profiles of Test drug batches compared to Reference batch 2356043.

dissolution profiles, a similar behavior was observed between the Reference and Test drugs, showing little point to point differences and similarity in form and complying with the difference and similarity factors f_1 and f_2.

Subjects

Twenty four healthy Colombian of both sexes were chosen for the present study, between 20 to 28 years old. The average weights and heights were 61.96 ± 10.23 kg, and 1.69 ± 0.10 m, respectively. No serious adverse event was observed during the study that impedes the participation of any subject. Three female volunteers and one man did not complete the study due to personal problems; however, the design was balanced in the number of subjects that took each kind of tablet, thus the statistic treatment could be made without affecting the result. Finally, the study was developed in twenty subjects, which represents a sample size higher than the recommendation for these studies [10,31].

Validation of the bioanalytical method

The method was selective, since in the analysis of six samples of plasma blanks of different origin there was no interference between the analyte and the endogenous matrix compounds. The Escitalopram in plasma calibration curve showed linearity in the concentration range of 1 to 200 ng/ml, with a correlation coefficient of 0.9992. The detection limit for the study was 0.0670 ng/mL and the quantification limit was 1.0 ng/mL. The precision expressed in the coefficients of variation intraday was 9.52%, 5.82% and 1.73% for 5 ng/ml, 15 ng/ml and 25 ng/ml respectively, and interday was 4.40%, 7.80% and 2.91% for 5 ng/ml, 15 ng/ml and 25 ng/ml respectively. As to recovery method, 90.9%, 61.6% and 65.6% was obtained for 5 ng/ml, 15 ng/ml and 25 ng/ml respectively. Escitalopram showed stability to the different conditions proposed by the FDA [20].

Pharmacokinetic and statistical analysis

The mean of the pharmacokinetic profiles for Escitalopram plasma concentration vs. time of the 20 volunteers is shown in Figure 2 and the mean pharmacokinetic parameters are described in Table 3. The ANOVA results showed that there are no statistically significant differences for the variables: sampling periods (F = 0.167 < F_{crit} = 4.098, P value= 0.685>0.05), and individual area calculation for each treatment (F = 1.191 < F_{crit} = 4.098, P value= 0.282 > 0.05), in terms of $LogC_{max}$ and $LogAUC_{0 \rightarrow 96}$. The 90% confidence interval of the average ratio $LogC_{máx}$ and $LogAUC_{0 \rightarrow 96}$, with 80% power, between the Test and Reference products for the 20 volunteers is shown in Table 4.

Discussion

Twenty volunteers completed the study; mild adverse reactions such as dizziness and nausea were presented, but were successfully controlled.

A bioanalytical method for plasma Escitalopram quantification by UHPLC-MS was validated, which showed to be selective, specific, precise and accurate for the work range, obtaining results that comply with the acceptance criteria defined in the international guidelines. On the other side, it was shown that Escitalopram is stable in plasma during sampling process at room temperature for 6 hours, in the storage at -80ºC, during freezing and thawing cycles and in the autosampler after processing the sample, complying with the FDA specification where it is emphasized that the coefficients of variation and percentages of error should be less than 20% [20].

The Pharmacokinetic values found for $AUC_{0 \rightarrow t}$, C_{max}, K_e, T_{max}, $T_{1/2}$,

Product	Assessment of the active ingredient %DV	Uniformity of dosage units %DV		Dissolution Test HCl 0.1 N % Dissolved after 30 min
Reference 2356043	102.6	101.7	L1 = 11.37	98.85
Test 3K2642A	105.1	102.8	L1 = 9.37	101.70
Test 4A0529	---	---		104.65
Test 3P3479B	---	---		102.62
Specification	%DV: 90.0-110.0%	%DV: 90.0-110.0%	L1≤15.0	Mean % dissolved after 30 min should not be less than the 80% of the declared amount
Result	Comply	Comply		Comply

Table 2: Results of controls of active ingredient content, uniformity of dosage units and dissolution tests for the Reference and Test drug batches.
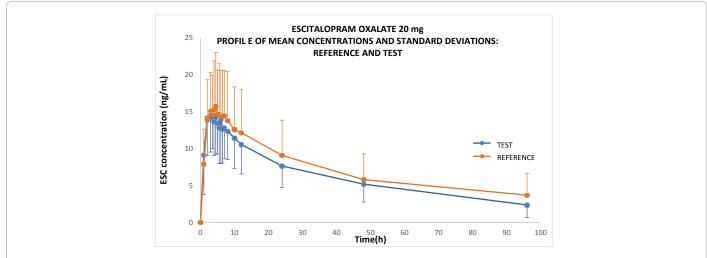

Figure 2: Mean of the pharmacokinetic profiles of the Escitalopram plasma concentrations vs. time from 0 to 96 hours of 20 volunteers, following a dose administration of the Test and Reference products of Escitalopram 20 mg tablets.

Parameters	Mean ± SD	
	Test Product	Reference Product
T_{max} (h)	3.0 ± 1.9	4.5 ± 2.3
C_{max} (ng/ml)	14.5 ± 5.9	15.7 ± 7.3
$AUC_{0\to96}$ (ng.h/ ml)	612.7 ± 207.6	740.9 ± 354.0
$AUC_{0\to\infty}$ (ng.h/ml)	731.3 ± 257.1	901.6 ± 389.2
K_e (1/h)	0.020 ± 0.004	0.023 ± 0.002
$t_{1/2}$ (h)	32.7 ± 4.62	33.9 ± 4.79

Table 3: Mean of pharmacokinetic parameters of Escitalopram tablets following administration of a 20mg dose of Test and Reference products in 20 volunteers.

Parameter	Ratio Test product/Reference product	90% Confidence interval
$LogC_{max}$ (ng/ml)	99.5	91.7-107.4
$LogAUC_{0\to96}$ (ng.h/ml)	98.8	94.6-103.1

Table 4: 90% Confidence intervals and mean of the $LogC_{max}$ and $LogAUC_{0\to96}$ rate between the Escitalopram 20 mg Test and Reference products tablets.

and $AUC_{0\to\infty}$ are very similar between reference and test products (Table 4). Also the Test/Reference product rates for $LogAUC_{0\to t}$ and Log C_{max} were 98,8% and 99.5% respectively, with 90% confidence intervals of significance level of 0.05 of 94.6-103.1% and 91,7 – 107,4 %, both being included in the bioequivalence region (80-125%) according to international standards [11]. These results were supported by ANOVA analyses that showed no statistical differences between the areas estimation for reference and test products, and no statistical differences between the two sampling periods.

The Bioequivalence study between the Escitalopram 20 mg tablets made by Laboratorios Tecnoquímicas S.A. (Test product) and Lexapro* 20 mg made by H. Lundbeck A/S (Reference product) was successfully developed in 20 healthy volunteers, who were administered with a single dose of each study formulation. It also allowed to compare the pharmacokinetic parameters values obtained with the values published in scientific articles related to the pharmacokinetic, and/or Bioavailability of this drug [4-6,26,31] observing similar behaviors of the area under the curve, maximum concentration, time to reach the maximum concentration, half- life time, etc., parameters (Table 3).

Although in drugs of long half-lives parallel designs are recommended, we used a crossover design because it has more precision and efficiency. As each participant receives both treatment and control, every participant serves as his own control, increasing the efficiency. This also means comparisons are based on within- subjects and not between subjects, which lowers the interferences of individual metabolism and increase precision. We used a longer washout period to

avoid the carry-over effect, taking only the advantages of the crossover design.

The sample size was chosen following the guidelines of the Instituto Nacional de Vigilancia de Medicamentos y Alimentos (INVIMA), which suggests a minimum number of 12 patients, and other bibliographical references that suggest that the real sample size never should be less than 12 volunteers [10,31]. Following this guides, the enrollment of twenty volunteers was appropriate for the study. However, information on intersubject variability on this and other studies show a coefficient of variance of 0.30 to 0.40. This means, according to Marzo and Balant equation [31], that sample size should be between 45 and 64 participants. Consequently this study could be interpreted as an early approximation. However as was discussed above, the results are consistent, and literature shows similar values of those we found. For these reasons, it can be concluded that the Test formulation Escitalopram 20 mg coated tablets by Laboratorios Tecnoquímicas S.A., is interchangeable or Bioequivalent to the Reference formulation Lexapro® 20 mg coated tablets by H. Lundbeck A/S.

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