Bioequivalence of zolpidem hemitartrate: new formulations of 5 mg SL, 10 mg and 12.5 mg XR and comparison of their bioavailabilities

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ABSTRACT
Objective: To evaluate pharmaceutical bioequivalence between two formulations of 5 mg zolpidem hemitartrate sublingual and, 10 and 12.5 mg extended-release formulations tablets in healthy subjects under fasting and fed conditions. Methods: An open label, monocentric, randomized, 2 x 2 crossover study in 40 healthy adults under fasting conditions comparing two formulations of zolpidem 5mg sublingual tablets. Analyte concentrations in human plasma were determined using a validated liquid chromatography with a tandem mass spectrometer detector method (LC-MS/MS). The same design was utilized to evaluate the other formulations. Results: Statistical analysis has determined geometric mean of test / reference ratio, confidence intervals, and power of the test to the pharmacokinetic parameters C_{max}, $AUC_{0-t,}$ and $AUC_{0-\infty.}$ The geometric mean ratio (90%CI) of the test drug/reference drug for zolpidem 5mg were 99.89 to 113.57% for C_{max}, 97.15% to 108.40% for AUC_{0-t}, and 97.22% to 108.13% for $AUC_{0-\infty.}$ Power of the test was 99.35% for C_{max} and 100% AUC_{0-t}, and $AUC_{0-\infty.}$ Conclusion: Both test and reference are bioequivalent for all formulations and, therefore, they are interchangeable, according to the Brazilian criteria (Anvisa resolution RE nº 1170/2006), since confidence intervals for C_{max} and AUC_{0-t} ratios were within 80% and 125%.

Keywords: zolpidem, bioequivalence, sublingual tablet, LC-MS/MS, hypnotic.

RESUMO
Objetivo: Avaliar a bioequivalência farmacêutica entre duas formulações de hemitartrato sublingual de zolpidem 5 mg e comprimidos de formulações de libertação prolongada de 10 e 12,5 mg em indivíduos saudáveis em jejum e sob condições de alimentação. Métodos: Um estudo cruzado, monocêntrico, aleatorizado, aberto, de 2 x 2 em 40 adultos saudáveis em jejum, comparando duas formulações de comprimidos sublinguais de zolpidem 5 mg. As concentrações do analito no plasma humano foram determinadas por cromatografia líquida validada com um método de detecção por espectrómetro de massa em tandem (LC-MS/MS). O mesmo desenho foi utilizado para avaliar as outras formulações. Resultados: A análise estatística determinou a média geométrica da razão teste/referência, os intervalos de confiança e a potência do teste em relação aos parâmetros farmacocinéticos Cmax, AUC0-t e AUC0- ∞. A razão média geométrica (IC90%) do fármaco de teste/fármaco de referência para zolpidem 5 mg foi de 99,89 a 113,57% para a Cmax, 97,15% a 108,40% para a AUC0- t e 97,22% a 108,13% para a AUC0- ∞. O poder do teste foi de 99,35% para a Cmax e 100% para a AUC0- t e para a AUC0- ∞. Conclusão: Tanto o teste quanto a referência são bioequivalentes para todas as formulações e, portanto, são intercambiáveis, de acordo com os critérios brasileiros

(resolução RE nº 1170/2006 da Anvisa), uma vez que os intervalos de confiança para as taxas Cmax e AUC0-t estavam entre 80% e 125%.

Palavras-chave: zolpidem, bioequivalência, comprimido sublingual, LC-MS/MS, hipnótico.

RESUMEN
Objetivo: Evaluar la bioequivalencia farmacéutica entre dos formulaciones de 5 mg de hemitartrato de zolpidem sublingual y de 10 y 12,5 mg de comprimidos de liberación prolongada en sujetos sanos en ayunas y alimentados. Métodos: Estudio abierto, monocéntrico, aleatorizado, 2 x 2, cruzado en 40 adultos sanos en ayunas, en el que se compararon dos formulaciones de comprimidos sublinguales de zolpidem de 5 mg. Las concentraciones de analito en el plasma humano se determinaron mediante cromatografía líquida validada con un método de detector de espectrómetro de masas en tándem (LC-MS/MS). El mismo diseño se utilizó para evaluar las otras formulaciones. Resultados: El análisis estadístico ha determinado la media geométrica de la relación prueba / referencia, los intervalos de confianza y la potencia de la prueba con respecto a los parámetros farmacocinéticos Cmax, AUC0-t y AUC0-∞. La razón media geométrica (IC del 90%) del fármaco de prueba/fármaco de referencia para zolpidem 5mg fue de 99,89 a 113,57 % para Cmax, de 97,15 % a 108,40 % para AUC0-t y de 97,22 % a 108,13 % para AUC0-∞. La potencia de la prueba fue del 99,35% para Cmax y 100% AUC0-t, y AUC0-∞. Conclusión: Tanto la prueba como la referencia son bioequivalentes para todas las formulaciones y, por lo tanto, son intercambiables, de acuerdo con los criterios brasileños (resolución Anvisa RE n.º 1170/2006), ya que los intervalos de confianza para las relaciones Cmax y AUC0-t estaban dentro del 80% y el 125%.

Palabras clave: zolpidem, bioequivalencia, tableta sublingual, LC-MS/MS, hipnótico.

1 INTRODUCTION

Zolpidem is, at present, the most frequently prescribed hypnotic drug in most countries. According to a current survey by the International Narcotics Control Board (INCB), Brazil consumed 4 tons of zolpidem for clinical and scientific purposes in 2022[1, 2].

Zolpidem hemitartrate is a hypnotic agent of the imidazopyridines group, used for the treatment of short-term insomnia. The formulation of zolpidem as an oral sublingual disintegration tablet was developed to provide a faster onset sleep than the oral immediate release formulation. Zolpidem pharmaceutical sublingual formulation proved more effective than an equivalent oral dose of zolpidem with regard to sleep induction[3-6]. Some drugs, such as carbamazepine, ciprofloxacin and fluvoxamine, have pharmacokinetic interaction with zolpidem[7-9].

The drug was synthesized by Synthelabo Recherche in the early 80s and proved to be an adequate and well tolerated product, especially with respect to the efficacy for sleep onset[10]. The FDA (Food and Drug Administration, USA) issued a letter of approval of the first tablet containing zolpidem tartrate in 1992 for Lorex Pharmaceuticals [11]. In Brazil, the

first registry of an extended-release coated tablet containing zolpidem was approved in 08/20/2007 by the Brazilian regulatory agency for Stilnox product. In 2009, the FDA issued a letter of approval for Edluar, the first 5mg and 10 mg zolpidem tartrate sublingual tablet. In 2011, ANVISA published the record of PATZ SL® (5mg and 10mg zolpidem hemitartrate sublingual tablet) which, since 2012 appears as reference drug in the agency list[11-13].

Studies reported by other authors show that zolpidem is well tolerated and that adverse events are mainly of the central nervous system and gastrointestinal, of mild to moderate severity grade[4, 14, 15]. According to pharmacovigilance data, ANVISA was notified of 189 adverse events suspected to be related to the administration of zolpidem and zolpidem hemitartrate in the period between 01/01/2018 and 07/31/2022, out of which 110 were related to psychiatric disorders, and 18 to gastrointestinal disorders, consistent with the publications of the above mentioned authors; however, other adverse events were also reported with less frequency, such as injuries, intoxications, and dermal tissue and musculoskeletal disorders[16]. Although the product has been reported as safe, two safety alerts have been issued by the FDA: one in 2013, related to changes in zolpidem insert and dose, and a recommendation to avoid driving the day after use, and the other in 2019, which required serious damage risk alerts due to somnambulism [17, 18].

The objective of the studies was to verify whether the formulations manufactured by Eurofarma Laboratórios S.A. (5 mg zolpidem hemitartrate sublingual tablet, 10 mg zolpidem hemitartrate coated tablet and 12.5 mg zolpidem hemitartrate extended-release multilayer tablet) have an absorption rate and extent equivalent to those of the respective reference formulations, PATZ SL®, 10 mg Stilnox®, and 12.5 mg Stilnox® CR, when administered alone and in one single dose under fast or fed conditions to healthy adult male and female subjects.

2 METHODS

2.1 STUDY FORMULATIONS

The test drug, 5 mg zolpidem hemitartrate sublingual tablet, 10 mg coated tablet, and multilayer 12 mg formulations were manufactured by Eurofarma Laboratórios S/A. The reference drug used in the 5 mg study was PATZ SL® (5 mg zolpidem hemitartrate sublingual tablet), manufactured by Novamed Fabricação de Produtos Farmacêuticos Ltda and registered by EMS Sigma Pharma Ltda. The reference for 10 mg presentation was Stilnox®, manufactured by Sanofi-Aventis Farmacêutica Ltda. The reference for 12 mg formulation was Stilnox® CR (Controlled Release), from Sanofi-Aventis Farmacêutica Ltda.

2.2 STUDY SUBJECTS

Forty subjects -20 male and 20 female- were selected for the study, between 18 and 50 years of age and BMI in the range of 18.50 to 28.63 kg/m², with no restrictions as to the ethnic group. The selected subjects were informed on the trial and, after having their inquiries clarified and having decided to willingly take part in the study, each subject signed the Informed Consent Form (ICF) approved by the Ethics and Research Committee of the Pharmaceutical Science Institute, along with the study protocol.

Initially, 40 subjects were selected and randomized according to the inclusion and exclusion criteria set forth in the protocol. However, due to exclusion and dropout reasons, only 38 volunteers completed the trial, 19 male and 19 female.

2.3 STUDY DESIGN

The bioequivalence study was monocentric, open-label, randomized, prospective, crossover (crossover 2x2), two-period, two-treatment (test drug = T and reference drug = R), two-sequence (RT and TR), balanced, single-dose, to compare to formulations of 5 mg zolpidem hemitartrate sublingual tablet in adult healthy male and female subjects under fasting conditions.

2.4 DRUG ADMINISTRATION

In each period, subjects were orally administered a 5mg zolpidem hemitartrate dose, corresponding to either 1 sublingual tablet of the test drug or 1 sublingual tablet of the reference drug, according to the randomization list and after a minimum period of 8 hours under fasting conditions, as required by Anvisa (*Agência Nacional de Vigilância Sanitária*)[19]. Drug administration was performed directly under the tongue of each subject, who kept the drug in that position for at least 5 minutes, the ingestion of any remains of the drug being allowed only thereafter. Subjects remained without any fluid intake for 2 hours and seated for 4 hours after administration. Washout period was 7 days, in compliance of the minimum period of seven half-lives of drug elimination.

2.5 BLOOD SAMPLING

Twenty-two blood samples of each subject were collected in each period, at timepoints before administration -01:00 (baseline) and 0:05, 0:10, 0:15, 0:20, 0:25, 0:30, 0:40, 0:50, 01:00, 01:15, 01:30, 02:00, 02:30, 03:00, 04:00, 05:00, 06:00, 08:00, 12:00, 16:00 and 24:00, and after

administration, in tubes with EDTA anticoagulant. The study subjects were discharged after the 24-hour collection.

2.6 BIOLOGICAL SAMPLES PROCESSING

Immediately after collection, all tubes were centrifuged at 3000 rpm for 5 minutes at approximately 4ºC, in a yellow light room, minimizing UV ray exposure. Plasma was split in 2 tubes, each of which with approximately 2.0 mL of plasma, and stored in an ultra-low temperature freezer (-70ºC e -80ºC).

2.7 ZOLPIDEM QUANTIFICATION IN HUMAN PLASMA

2.7.1 Method Validation

Bioanalytical method validation for the quantification of zolpidem in human plasma with EDTA as anticoagulant, using zolpidem-d7 as internal standard and through extraction by protein precipitation and liquid chromatography coupled to mass spectrometry was performed in compliance with the acceptance criteria for selectivity, calibration curve, precision, accuracy, residual effect, matrix effect and stability tests in the solution and in the biological matrix set forth by the Brazilian regulatory agency legislation[20].

The method proved linear between concentrations of 1.00 ng/mL to 400 ng/mL according to equation y = a + bx [1/x], where “y” is the response, “x” is the analyte concentration and “1/x” is the selected weight.

The lower limit of quantification (LLQ) set for the method was of 1.00 ng/mL and the validated quality control samples were 3.00 ng/mL, 160 ng/mL and 320 ng/mL.

2.7.2 Stability

Stability tests were performed in plasma in concentrations of t 3.000 ng/mL and 320.000 ng/mL and they complied with the acceptance criteria when the samples were subjected to 17 hours at room temperature before the sample extraction process (short-term stability), 115 hours stored in the autoinjector at room temperature after completing sample extraction (post-processing stability) 3 freeze and thaw cycles and 119 long-term days.

2.7.3 Standard Solutions and Reagents

The reagents used included ultrapure water obtained through Millipore purification equipment, methanol HPLC grade (Merck), 30% ammonia solution analytical grade (Sigma Aldrich) and ammonia acetate analytical grade (Merck).

Reference standard zolpidem tartrate (United States Pharmacopeia, USA) was used as analyte and zolpidem-d7 (Cerilliant, USA) as internal standard for the preparation of the primary standard solutions in methanol/water (80/20; v/v).

2.7.4 Compounds Quantification in Biological Samples

Compounds were extracted from human plasma samples and quantified by means of a liquid chromatography coupled to mass spectrometry (LC-MS/MS) using an API 5000 (Sciex / Applied Biosystems) spectrometer, equipped with positive electrospray ionization source (ESP+) and detecting analyte (Zolpidem) and internal standard (Zolpidem-D7) using multiple reactions mode (MRM) with mass to charge ratio (m/z) transitions 308.3>263.2 and 315.2>270.2, respectively.

2.7.5 Softwares Used

Analyst version 1.4.2 was used for calculating sample concentrations in the analytic phase.

WinNonlin™ and Microsoft Office suite were used for performing the statistical analysis.

2.7.6 Other Studies Sponsored

Bioequivalence studies were also conducted with two other formulations: 10 mg coated tablets and 12.5 mg extended-release multilayer tablets, the latter being tested under two conditions: fasting and fed. The designs and results of these studies are presented below.

3 RESULTS

3.1 5 MG SUBLINGUAL TABLET FORMULATION

3.1.1 Study Population

The study was completed with 38 adult healthy subjects between 19 and 50 years of age, and with a body mass index (BMI) between 20.93 and 28.56 kg/m², and according to the inclusion and exclusion criteria set forth in the protocol.

3.1.2 Pharmacokinetics and Statistical Analysis

Pharmacokinetic parameters C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ were set using *WinNonlin*™ software and Microsoft Office.

Pharmacokinetic parameters are shown in Table 1.

Table 1. Geometric means, confidence intervals and p-values obtained in the study analysis for 5mg sublingual tablets formulations, and 10 mg coated tablet in fasting conditions.

Statistical Results for Pharmacokinetic Parameter for 5mg SL tablet			
	C_{max} (ng/mL)	**AUC_{0-t} (ng.h/mL)**	**$AUC_{0-\infty}$ (ng.h/mL)**
	Geometric means by least square method		
5 mg Patz SL® (Reference)	93.9679	415.8698	430.0797
5 mg Zolpidem (Eurofarma)	100.0834	426.7770	440.9563
	Intervals of Confidence (Shortest) obtained for the ratio between treatments (transformed data)		
Contrast	IC_90%	IC_90%	IC_90%
Test vs Reference	99.89% - 113.57%	97.15% - 108.4%	97.22% - 108.13%
	Specific estimates obtained for treatment ratio (%)		
Test/Reference	106,51	102,62	102,53
	***A posteriori* test power (%) – calculated using TOST method (%)**		
Test/Reference	99.35	100.00	100.00

Statistical Results by Pharmacokinetic Parameter for 10 mg coated tablet			
	C_{max} (ng/mL)	**AUC_{0-t} (ng.h/mL)**	**$AUC_{0-\infty}$ (ng.h/mL)**
	Geometric means by least squares method		
10 mg Stilnox ® (Reference)	170.1277	578.9283	587.9075
10 mg Zolpidem (Eurofarma)	178.9199	961.2880	573.1792
Test/Reference Ratio	105.17	96.95	97.49
	Intervals of Confidence (Shortest) obtained for the ratio between treatments (log-transformed data)		
Contrast	IC_90%	IC_90%	IC_90%
Test vs Reference	91.46% - 120.93%	91.2% - 103.07%	91.89% - 103.45%
	Specific estimates obtained for treatment ratio (%)		
Test/Reference	105.17	96.95	97.49
	***A posteriori* test power (%) – calculated by TOST method (%)**		
Test/Reference	60.33	99.98	99.99

Source: Own Authorship.

Maximum concentration C_{max} obtained for the reference product PATZ SL® of 98.753 ng/mL occurred in 0.891 hour. For the test drug, zolpidem hemitartrate, the 105.737 C_{max} occurred in 0.919 h.

Figure 1 (A) shows the average concentrations of zolpidem for the 38 subjects along collection timepoints obtained with the 5mg sublingual formulations.

3.1.3 Tolerability and Safety Analysis

Thirty-seven adverse events were reported during the study, of which one was moderate (allergic reaction) and the others were mild.

As to causality, only 3 adverse events were classified as suspected of being related to the drug. No serious adverse events were reported.

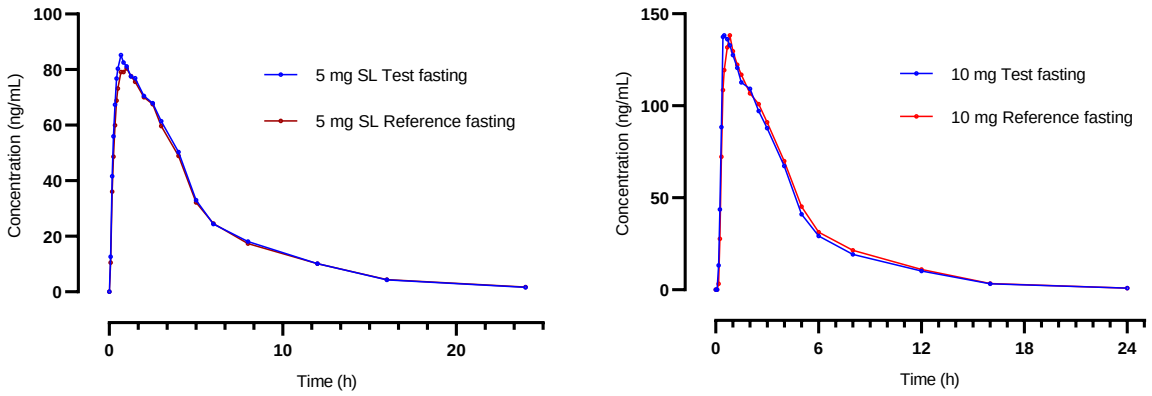
The most common adverse event was leukocyturia, which represented 28% of the total events reported by the subjects who constitute the safety population. Said adverse event was classified as not suspected to be related to the administered drug, and was therefore characterized as an event which may occur due to other physiologic conditions. Adverse events classified as suspected to be related with the drug were glutamic oxaloacetic transaminase (GOT) serum raise, allergic reaction and muscular weakness.

3.1.4 10 mg Coated Tablets Formulation

The study was completed with 38 adult healthy subjects between 18 and 50 years of age, and a body mass index (BMI) between 18.50 and 28.63 kg/m², and according to the inclusion and exclusion criteria set forth in the protocol. According to the previously approved protocol, the study was monocentric, open-label, crossover, randomized, prospective, two-treatment, two-period, two-sequence and designed to initially include 40 healthy male and female study subjects, between 18 and 50 years of age who, in each period, were administered either the test drug, i.e., 10 mg Zolpidem coated tablet (manufactured by Eurofarma Laboratórios S.A.) or the reference product 10 mg Stilnox® (manufactured by Sanofi-Aventis Farmacêutica Ltda.) according to the randomization list. Collections were conducted in a tube with EDTA at timepoints: baseline (-01:00)/ 00:05 / 00:10 / 00:15 / 00:20 / 00:25 / 00:30 / 00:40 / 00:50 / 01:00 / 01:15 / 01:30 / 02:00 / 02:30 / 03:00 / 04:00 / 05:00 / 06:00 / 08:00 / 12:00 / 16:00 / 24:00 hours after administration. The obtained pharmacokinetic parameters are shown in Table 1.

Figure 1. (A) Average zolpidem concentrations through time for each formulation, test and reference, for the 5mg SL (sublingual) formulation, in fasting conditions. (B) Average zolpidem concentrations through time for each formulation, test and reference, for the 10 mg coated tablets formulation, in fasting conditions.
Figure 1 (B) shows average zolpidem concentrations for the 38 study subjects through collection times obtained with the 10 mg coated tablets.

(A) (B)



Source: Own Authorship.

3.1.5 12.5 mg Coated Tablets Formulation Under Fasting and Fed Conditions

The study was completed with 37 adult healthy subjects between 18 and 49 years of age, and a body mass index (BMI) between 19.03 and 29.90 kg/m², and according to the inclusion and exclusion criteria set forth in the protocol. According to the previously approved protocol, the study was monocentric, open-label, crossover, randomized, prospective, two-treatment, two-period, two-sequence and designed to initially include 40 healthy male and female study subjects, between 18 and 50 years of age who, in each period, were administered either the test drug, i.e., 12.5 mg Zolpidem extended-release tablet (Eurofarma Laboratórios S.A.) or the reference product 12.5 mg Stilnox® CR (Sanofi-Aventis Farmacêutica Ltda.) according to the randomization list. Collections were conducted in a tube with EDTA at timepoints: -01:00/ 00:15 / 00:30 / 00:45 / 01:00 / 01:15 / 01:30 / 01:45 / 02:00 / 02:15 / 02:30 / 02:45 / 03:00 / 03:30 / 04:00 / 05:00 / 06:00 / 08:00 / 10:00 / 12:00 / 14:00 / 24:00 hours after administration. The obtained pharmacokinetic parameters are shown in Table 2.

Table 2. Geometric means, confidence intervals and p-values obtained in the study analysis for 12.5 mg extended-release multilayer tablets under fasting and fed conditions

Statistical Results for Pharmacokinetic Parameter 12,5 mg extended-release (fasting)			
	C_{max} (ng/mL)	**AUC_{0-t} (ng.h/mL)**	**$AUC_{0-\infty}$ (ng.h/mL)**
Geometric means using least squares method			
12.5 mg Stilnox® CR (Reference)	170.791	1023.746	1066.130
12.5 mg Zolpidem (Eurofarma)	163.707	997.223	1033.315

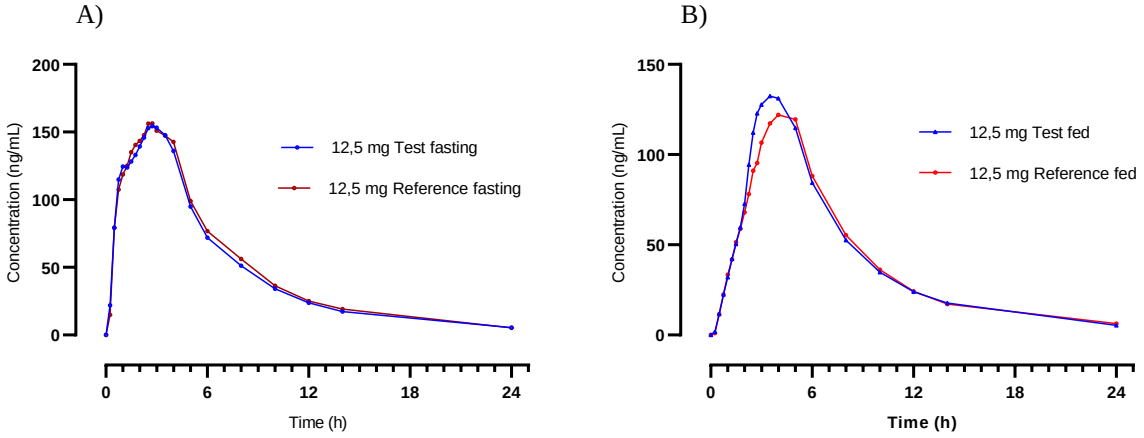
Test/Reference Ratio	95.85	96.65	96.92
Intervals of Confidence (Shortest) obtained for the ratio between treatments (log-transformed data)			
	IC_90%	IC_90%	IC_90%
Test vs Reference	90.02% - 102.06%	91.09% - 102.56%	91.20% - 103.01%
	***A posteriori* test power (%) – calculated using TOST method (%)**		
Test/Reference	99.99	100.00	100.00

Statistical Results for Pharmacokinetic Parameter 12,5 mg extended-release (fed)			
	C_{max} (ng/mL)	**AUC_{0-t} (ng.h/mL)**	**$AUC_{0-\infty}$ (ng.h/mL)**
	Geometric means using least squares method		
12.5 mg Stilnox® CR (Reference)	131.769	837.585	885.383
12.5 mg Zolpidem (Eurofarma)	143.208	843.931	879.430
Test/Reference Ratio	108.68	100.76	99.33
Intervals of Confidence (Shortest) obtained for the ratio between treatments (log-transformed data)			
	IC_90%	IC_90%	IC_90%
Test vs Reference	101.52% - 116.35%	94.41% - 107.53%	92.39% - 106.79%
	***A posteriori* test power (%) – calculated using TOST method (%)**		
Test/Reference	99.98	99.99	99.94

Source: Own Authorship.

The Figure 2 shows average concentrations of zolpidem for the 37 study subjects obtained through collection times with 12.5 mg extended-release tablets formulation.

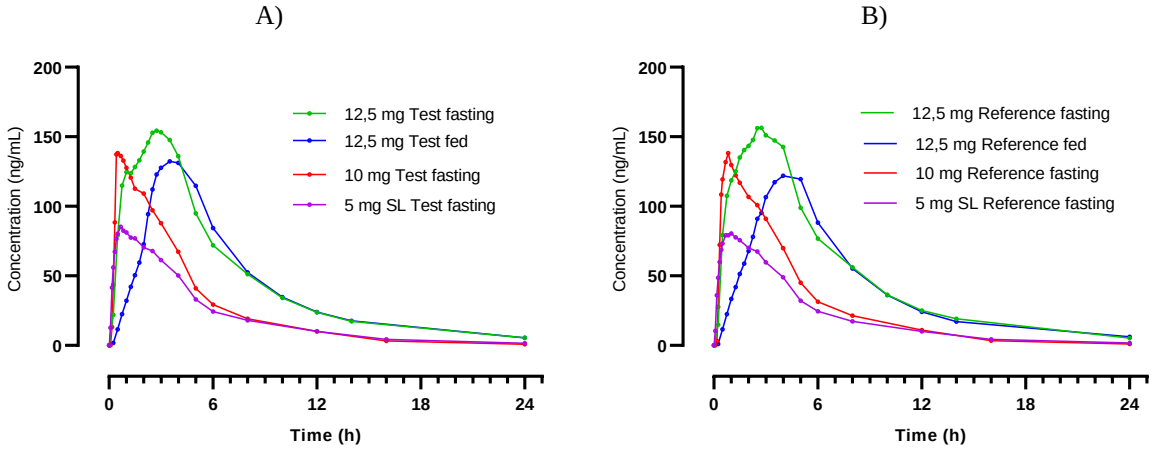
Figure 2. Average zolpidem concentrations through time for each formulation, test and reference, for the 12,5 mg (extended-release tablets) under fasting (A), and fed (B) conditions.



Source: Own Authorship.

The Figure 3 shows, the average concentrations of zolpidem for study subjects obtained through collection times with all the formulations studied.

Figure 3. Average concentrations of zolpidem through time for each formulation, test (A) and reference (B) comparing the profiles of 5 mg SL tablet, 10 mg coated tablet and 12.5 extended-release tablet formulations under fasting condition, and 12.5 extended-release tablet formulation under fed condition.



Source: Own Authorship.

4 DISCUSSION

Insomnia has different symptoms such as the difficulty to fall sleep, difficulty to stay asleep, early waking up, difficulty to get back to sleep after waking up or a combination of two or more of those symptoms[21].

In clinical practice, the choice of a sleep-inducing drug is based on the patient sleep standard. Those who have difficulty just for falling asleep benefit from fast release formulations such as a sublingual tablet; on the other hand, those who wake up several times through the night benefit more from extended-release formulations which maintain the pharmacodynamics effect for a longer period[22, 23].

Those differences in needs, especially the duration of the pharmacodynamics effect, direct the development of formulations which present differentiated pharmacodynamics profiles as shown in Figure 3, as the drug shows a direct pharmacokinetic/pharmacodynamics relationship[24]. Therefore, having an arsenal which covers all situations, the physician may manage symptoms in a personalized and precise manner, optimizing treatment and possibly minimizing adverse events, especially in more susceptible populations to such effect, as elderly or pediatric patients[25-27].

The results obtained for the 5 mg sublingual formulation agree with those already stated in the literature[22]**.** On the other hand, the results obtained for the 10 mg and 12.5 formulations are unedited in relation to bioequivalence in the literature.

The studies conducted enrich the therapeutic arsenal for insomnia in order to treat the many different varieties of the disease with generic formulations which are more affordable for the patient and equivalent to the reference products in terms of quality and efficacy.

4.1 5 MG SL FORMULATION STUDY

The study was properly planned and conducted, and pharmacokinetic parameters C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ were obtained, the interval of confidence values of which (90%) are within the acceptable limit for the ratio between the geometric means of the test and reference products (80-125%), in accordance with the Brazilian legislation[28].

Forty study subjects were to participate in this study in order to meet the requirements of nominal significance level of 50 %, 2x2 crossover design, 80-125% equivalence range, 28% maximum CV_{intra} for pharmacokinetic parameters, population ratio between 95-105% for treatment average plus an additional number of 8 volunteers to compensate possible losses due to dropout/exclusions. The planned number of subjects for the study is consistent with studies reported buy some authors[29-31] and higher than the number estimated by other[10, 32]. The study was completed with 38 subjects, an amount higher than the 32 estimated volunteers in case of loss of the 8 subjects considered in the sample size determination, fact which contributed to maintain the obtained test power for pharmacokinetic parameters near 100%.

Some authors state that there is a negative food effect of in zolpidem absorption and extent rate[33, 34]. The Brazilian regulatory agency sets forth that bioequivalence studies with drugs containing zolpidem hemitartrate must be conducted under fasting condition [19] , as well as most of available drugs as previous reported [35, 36]. Nevertheless, to prove the impact of food in the absorption of zolpidem, the 12,5 mg formulation was tested both fed and fasting conditions [34]. Thus, consistent with scientific findings and regulatory requirements, for the others formulations, the drug administrations were under fasting condition of at least 8 hours.

Formulations proved to be well tolerated and most reported adverse events were mild and related to changes of laboratory tests, which may indicate that they can be the consequence of other physiologic conditions. Only 3 events were suspected to be related to the study drug, of which muscular weakness is consistent with musculoskeletal disorders notified to ANVISA[16].

W*ashout period of* 7 proved adequate, as all baseline sample collections of the subjects of the second period had a concentration below the lower limit of quantification (LLQ).

As in other published works, the analytical selected technique of the study to quantify zolpidem in human plasma was LC-MS/MS [29, 37, 38].

Both reference and test products for 5 mg sublingual formulations showed a maximum plasma concentration C_{max} of 98.753 ng/mL and 105.737 ng/mL, respectively, consistent with the values found by other authors [29, 34].

4.2 STUDIES COMPARISON AMONG 5 MG SUBLINGUAL TABLET, 10 MG COATED TABLET AND 12.5 EXTENDED-RELEASE TABLET FORMULATIONS

As previously discussed, different clinical scenarios necessitate distinct pharmacokinetic profiles to manage multiple forms of insomnia. The comparison of all pharmacokinetic curves on the same graph (Figure 3) illuminates this fact. The physicians may prescribe either rapid-release formulations or extended-release formulations, depending on the type of insomnia their patients have.

5 CONCLUSION

Bioequivalence between test and reference formulations in each of the four studies has been proven, in terms of both absorption rate and extent, as the criteria required by the Brazilian regulatory authority has been complied with (IC 90% between 80 and 125% for C_{max} and AUC_{0-t}).

Thus, zolpidem hemitartrate test formulations manufactured by Eurofarma Laboratórios S/A and reference formulations were bioequivalent and, therefore, interchangeable:

- 5 mg sublingual tablet and 5 mg Patz SL®;
- 10 mg coated tablet and 10 mg Stilnox®;
- 12,5 mg extended-release multilayer tablet and 12.5 Stilnox CR®

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