

Bioavailability Comparison of Two Zopiclone Formulations in Healthy Colombian Volunteers

Adriana Ruiz^{1*}, Fanny Cuesta², Paula Castaño³, Omar Correa¹, Karina Gómez¹ and Maria Elena Jaramillo¹

¹Department of Pharmacy, Faculty of Pharmaceutical Chemistry, University of Antioquia, Medellín, Colombia

²Department of Pharmacology, Faculty of Medicine, University of Antioquia, Medellín, Colombia

³IPS Universitaria, Clínica León XIII, Medellín, Colombia

Abstract

Zopiclone is a hypnotic short-acting agent used in the treatment of primary insomnia. The aim of this study was to compare the Bioequivalence of two formulations of Zopiclone available in the Colombian market: Zopiclone 7.5 mg commercialized as Zopiclone MK[®] and Zopicloteg TG[®] (Test product) manufactured by Tecnoquímicas S.A (Cali, Col.) and Imovane[®] (Reference product) from Sanofi-Aventis Farmacéutica Ltda (Brasil). With this purpose, a single dose, randomized, crossover, with two periods, two sequences and a washout period of one week study was developed. Blood samples were drawn from 0 to 24 hours following the drug administration. Zopiclone plasma levels were determined by HPLC method, validated under the FDA parameters. The 90% confidence intervals for the ratios of the $\ln AUC_{0-\infty}$ and $\ln C_{max}$ means between the Test and Reference product were constructed. The 80/125 rule was used as bioequivalence criterion. The study was conducted in 26 healthy volunteers. The estimated pharmacokinetic parameters for Zopiclone, either for the Test product or Reference product were C_{max} 72.815 ± 20.54 ng/mL, 74.315 ± 18.04 ng/mL; AUC_{0-t} 467.297 ± 92.21 ng.h/mL, 460.996 ± 115.81 ng.h/mL, and $AUC_{0-\infty}$ 560.298 ± 118.58 ng.h/mL, 543.549 ± 136.97 ng.h/mL, respectively. The 90% confidence intervals for the ratio between the averages of \ln -transformed data of $AUC_{0-\infty}$ and C_{max} were 97.38% - 110.59% and 89.97% - 104.84%, respectively.

Conclusion: In the present study of single dose, the Test product, Zopiclone 7.5 mg, meets the Bioequivalence criterion regarding the rate and extent of absorption.

Keywords: Zopiclone; Imovane[®]; Bioavailability; Bioequivalence; Pharmacokinetic; HPLC

Introduction

Zopiclone is a hypnotic short-acting agent whose chemical structure corresponds to a cyclopyrrolone derivative, which, although not chemically related to existing hypnotics, presents a pharmacologic profile similar to the Benzodiazepines group [1,2] by binding with high affinity to the pharmacological receptors of them [3].

Following its oral administration [4] it is quickly absorbed with an approximate bioavailability of 80%. Peak plasma levels are achieved 1.5 to 2.0 hours, with approximate peak levels of 30 and 60 ng/mL, following oral administration of 3.75 mg and 7.5 mg dose, respectively. Absorption is not modified by sex, food or dose repetition [5]. There is a drug interaction risk because it binds to plasma proteins of 45 to 80%. It is widely and quickly distributed to body tissues, including the brain. Its volume of distribution is 91.8 to 104.6 liters. It is partially metabolized by the liver into two metabolites, an inactive N-demethylated derivative and an active N-oxide derivative. It is excreted in the urine, saliva and breast milk. Additionally, about 50% of an administered dose is decarboxylated and eliminated by the lungs. Less than 7% of a dose is excreted via the urine as unchanged drug. The elimination half-life is within 3.5 to 6.5 hours. With the age, Zopiclone pharmacokinetics is affected due to variations in the liver. In Elderly patients a minimum dose should be administered since adverse reactions are dose-related. Zopiclone must be carefully administered to patients with drug abuse history [6].

Absorption of a drug and hence its bioavailability can be influenced by the composition and method of manufacture of the dosage form. Thus, a drug with the same dosage form made by different manufactures could present differences in the rate and extent of absorption; therefore, it is necessary to verify the bioavailability of a given drug from different formulations to the innovator product.

Considering the above, Tecnoquímicas S.A. asked to Grupo de Estudios e Investigaciones Biofarmacéuticas for the Bioequivalence determination of Zopiclone 7.5 mg tablets formulation commercialized as Zopiclone MK[®] and Zopicloteg TG[®], in relation to the innovator product Imovane[®]. With the results of the present study, the scientific bases for the therapeutic equivalence of the mentioned product will be obtained.

Subjects and Methods

Test products

The Test product was ZOPICLONE 7.5 mg tablets, manufactured and commercialized by Tecnoquímicas S.A (Cali, Colombia) as Zopiclone MK[®] and Zopicloteg TG[®], Batch H2036, provided by the manufacturing laboratory. The Reference product, IMOVANE[®] Zopiclone 7.5 mg, Batch 323392, made by Sanofi-Aventis Farmacéutica Ltda, was purchased in a local pharmacy.

Products underwent quality controls before the Bioequivalence study. Quality parameters such as drug content, dissolution and dosage-unit uniformity were tested, using analytical techniques given by the Test product manufacturer. These analysis were done in Laboratorio Especializado de Análisis of the Faculty of Pharmaceutical

***Corresponding author:** Adriana Ruiz, Professor, Department of Pharmacy, University of Antioquia, Medellín, Colombia, Tel: 5742195478; Fax: 5742195459; E-mail: amaria.ruiz@udea.edu.co

Received May 26, 2015; Accepted July 22, 2015; Published July 29, 2015

Citation: Ruiz A, Cuesta F, Castaño P, Correa O, Gómez K (2015) Bioavailability Comparison of Two Zopiclone Formulations in Healthy Colombian Volunteers. J Bioequiv Availab 7: 233-238. doi:10.4172/jbb.1000246

Copyright: © 2015 Ruiz A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Chemistry of the University of Antioquia. Products were considered appropriated to perform the Bioequivalence study if the drug content of the Test product did not differ more than 5% from the drug content of the Reference product; the average percentage dissolved of the drug from the tables, in 45 minutes, was not less than 80%, and the acceptance value for dosage-unit uniformity, performed on 10 tablets, was less than 15%.

Subjects

Healthy women and men between 18 and 30 years old, non-smokers and within the 15% of the ideal weight for height (Metropolitan Life Insurance Company Statistical Bulletin, 1983) [7] were considered as appropriate to participate in the study. The following aspects were taken into account as inclusion criteria: non-smoker subject, no consumer of abuse drugs, no history of acute or chronic disease, or evidence of liver, kidney or blood disorders; in other words, that the results of the paraclinical tests (complete blood count, alanine transaminase, aspartate transaminase, direct or indirect total bilirubin, serum creatinine) were within the range of the reference values established by international standards and with negative HIV and hepatitis B results. In addition, pregnancy test in women and alcohol and drug abuse tests in all volunteers had to be negative on dose administration days.

Subjects were informed by an investigator about the purposes and risks of the study, and written informed consent was obtained from all volunteers. They were asked to avoid taking prescription drugs, diet supplements and natural products 15 days before the first dose and throughout the end of the study. Products containing alcohol, caffeine and/or xanthines were forbidden 48 hours before the administration of the study products and during the sampling time. Subjects remained at fasting state for at least 12 hours before each dosing.

Study design

Both the protocol and the informed consent form were reviewed and approved by the Ethics Committee of the Faculty of Medicine of the University of Antioquia, Medellín, Colombia. The study was performed according to the Declaration of Helsinki [8], the Good Clinical Practice [9] and Resolution n° 8430 del Ministerio de la Protección Social, Republic of Colombia [10].

The study was conducted with a randomized, single-dose, two-treatment, two-period, two-sequence crossover design with one week washout period. According to a randomly assignment, in period I each volunteer received a tablet of the Reference or Test product, and in period II, they were administered a tablet of the product not received in the previous period.

Drug products administration and blood sampling

Subjects were confined in the hospital one-hour before Zopiclone administration, up to completing a sampling time of 24 hours. During the first hour, a urine sample was taken from each volunteer to confirm the absence of abuse drugs, alcohol and pregnancy in women. Vital signs were monitored and a catheter was placed into an antecubital vein; then, each subject received a Test or Reference product dose with 240 mL of water at room temperature.

Volunteers stayed at semi-sitting position for the following three hours after the drug administration to ensure adequate gastric emptying and during the confinement time, they remained at rest or wandering in reduced areas. Vital signs were monitored after dosing and measured again in volunteers that presented adverse reactions. Volunteers were under constant medical supervision, and during the

washout period they kept in communication with investigators to report any adverse event.

Volunteers had standardized meals, at 2.5, 5, 8.5, 11, 14 and 24.5 hours after the dosing (breakfast, lunch, snack, dinner, snack and breakfast the next day).

Blood samples were obtained at 0:00, 0:20, 0:40, 1:00, 1:30, 2:00, 3:00, 4:00, 6:00, 8:00, 12:00 and 24:00 hours. Blood was collected into heparinized vacutainer blood collection tubes (BD Franklin Lakes, NU, U.S.A.) and then it was centrifuged at 3000 rpm for 15 minutes. The plasma obtained was divided in three cryovials; one of them was frozen at -20°C until analysis, the others were stored at -190°C, in liquid nitrogen as retention samples.

Tolerability

The possible adverse reactions were monitored during both study periods and the washout period by interviews and reports of the subjects, who were asked to inform any undesirable sign or symptom occurring after the start of the study.

The medical staff classified the adverse reactions as mild (if they did not interfere with daily activities), moderate (if they interfere with daily activities but did not require drug discontinuation) or severe (if they put the volunteer at risk and require immediate medical intervention).

Determination of Zopiclone plasma concentrations

Plasma concentration of Zopiclone (racemic mixture) was determined by high performance liquid chromatography (HPLC), using clobazam as internal standard. The papers published by Paw and Misztal [11], Nirogi et al. [12] and the physicochemical properties of Zopiclone were taken as starting points in order to develop the quantitation method for this drug.

Individual samples were subjected to liquid-liquid extraction with toluene. After shaking they were centrifuged, the supernatant was evaporated under nitrogen stream followed by reconstitution with 200 µl of mobile phase and 100 µl were injected into a liquid chromatograph (model 1100, Agilent Technologies, Santa Clara, California) equipped with an Eclipse Plus C18 column (150 × 4.6 mm, 5 µm, Agilent Technologies, Santa Clara, California) at 30°C. A mixture of 20mM ammonia phosphate, pH 4.5 : Methanol (47:53) was used as mobile phase at a flow-rate of 1.0 mL/min. Detection was performed with an ultraviolet detector at wavelengths of 305 nm for Zopiclone, and 229 nm for clobazam (Figure 1).

The analytical procedure was validated according to the parameters established by international guidelines [13]. Quality control samples (10, 40 and 80 ng/mL) were included in the run of the samples of each volunteer to assess the method performance.

Pharmacokinetics and statistical analysis

A non-compartmental pharmacokinetic method was used to obtain the pharmacokinetics parameters. Plasma concentration-time curves for each product were constructed with the data obtained from each volunteer to obtain the maximum concentration (C_{max}) and time to reach it (T_{max}).

The elimination rate constant (k_e) was obtained by ln-linear regression of the terminal decay phase. Area under the plasma concentration-time curve from time zero to the last time point with quantifiable concentration (AUC_{0-t}), was determined using the trapezoidal rule. The total area under the plasma concentration-time

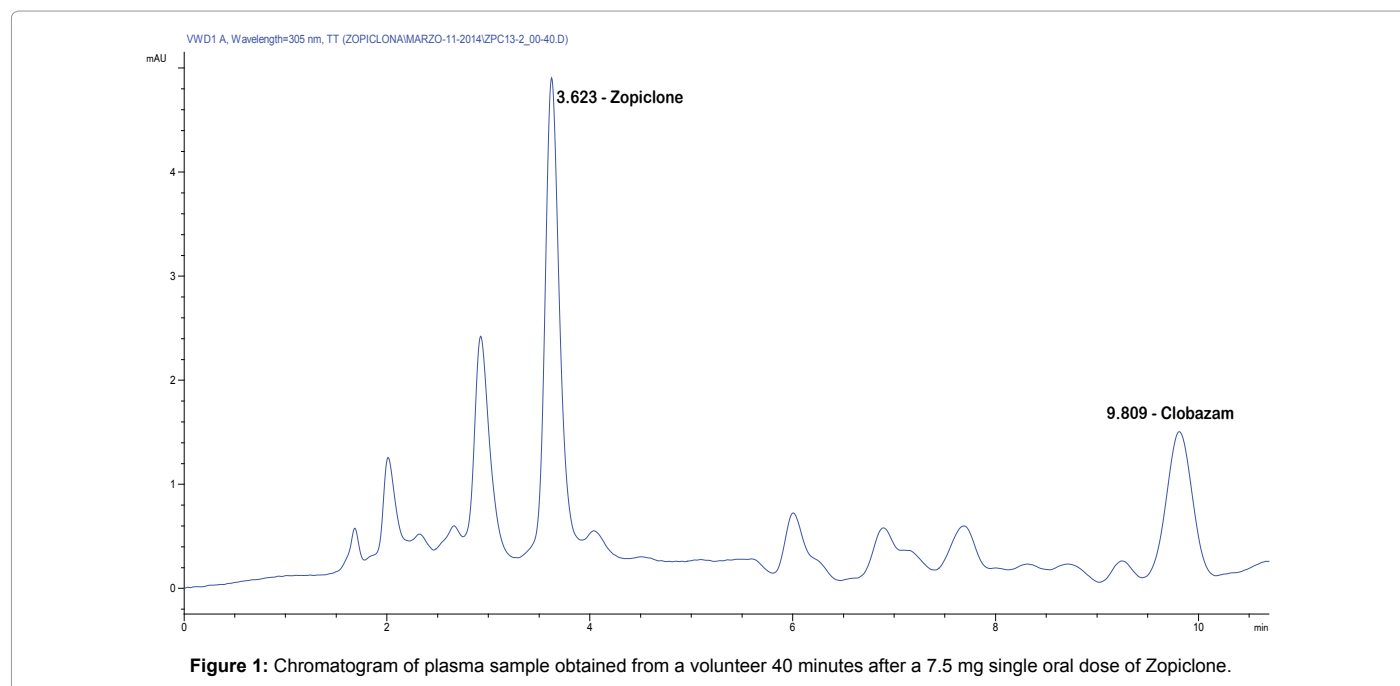


Figure 1: Chromatogram of plasma sample obtained from a volunteer 40 minutes after a 7.5 mg single oral dose of Zopiclone.

curve from time zero to infinity ($AUC_{0-\infty}$), was calculated by dividing the last plasma quantifiable concentration by k_e , and adding this result to AUC_{0-t} . Zopiclone plasma concentrations below the limit of quantification of the method were excluded from the calculations.

An analysis of variance (ANOVA) for the crossover design was performed on ln-transformed data of $AUC_{0-\infty}$ and C_{max} , in order to assess the effects of sources of variation such as treatment, period, and sequence.

The data were also analyzed in reference to the assumptions of two-treatment, two-period, two-sequence crossover design, in order to test: the carryover effects, the inter and intra subject variabilities, the inter and intrasubject residuals normality and residuals independence [14]. If a p-value was less than or equal to 0.05, the difference was considered as statistically significant. Potential outliers detection on untransformed data were carried out based on the Test minus Reference difference (t-r), Test/Reference ratio (t/r) and ln of the Test/Reference ratio (ln t/r) [15], using exploratory data analysis (box plot).

The two-one side method suggested by Schuirmann [16] and accepted by the FDA [17] was applied to conclude Bioequivalence, taking into account the standard error of the ANOVA for each parameter. The 90% confidence intervals for the ratios of the ln $AUC_{0-\infty}$ and ln C_{max} means between the Test and Reference products were constructed. Bioequivalence was concluded if these 90% confidence intervals lie in the bioequivalence range 80% to 125%.

Results

Products quality control

The results of drug content, dissolution, and dosage-unit uniformity for both products are shown in Table 1. The Test product showed a difference of 1% compared to the Reference product, for the drug content variable. Both products met the requirements for the tests.

Subjects

The study was developed in twenty-six (26) subjects, ten (10) men

Product	Drug content (%)	Dissolution (%)	Dosage-unit uniformity (%)
Test	98.2	95.2 ± 3.0	6.5
Reference	99.2	96.8 ± 1.7	4.6

Table 1: Results of drug content (mean percentage of drug per tablet), dissolution tests (mean percentage of drug dissolved ± standard deviation, n= 6) and dosage-unit uniformity test (acceptance value in percentage) of the Test and Reference products.

and sixteen (16) women aged between 18 and 30 years, with an average of 21.9 ± 2.4 years. Their weights varied between 44.0 and 78.3 kg, with an average of 60.5 ± 8.7 kg, and the height ranged from 1.49 to 1.81 m, with average of 1.66 ± 0.09 m.

Tolerability

In both periods, eight volunteers experienced bitter taste with both products. During the first period, with the Test product, a volunteer had vomiting at 1.25 hours after dosing, the administrated tablet was not found when the fluid was checking; another four developed hiccups, whereas with the Reference product two subjects experienced diarrhea and two more abdominal pains.

In the second period, two subjects experienced diarrhea; two developed dizziness and other two abdominal pains, with the Test product, and with the Reference product two developed symptoms of nausea and four experienced headaches.

Validation of the bioanalytical method

The analysis of six blank plasma showed a little interference co-eluted with Zopiclone, thus the blank chromatogram obtained for each volunteer was subtracted from the correspondent sample chromatograms. The same procedure was performed for calibration curves. No interfering peaks were observed for clobazam.

Calibration curves of spiked plasma samples were linear in the range of 5 to 100 ng/mL, with an average correlation coefficient of 0.9990 for interday (n=6) and 0.9994 for intraday (n=6) evaluations. The limit of quantification was 5.0 ng/mL. Zopiclone recovery ranged from 70.04% to 98.16%, with an average of 84.29%. Intraday accuracy for a low (10

ng/mL), medium (40 ng/mL) and high (100 ng/mL) concentration were 11.99%, 4.50% and 0.60%, respectively and interday accuracy for the same concentrations were 7.40%, 4.56% and 1.17%, respectively. For intraday precision, coefficients of variation were 12.40%, 4.84% and 0.64%, while for the interday precision were 8.83%, 4.06% and 0.69%. The results obtained from the stability study showed that Zopiclone in plasma was stable at -20°C during the period of analysis (four months).

Pharmacokinetics and statistical analysis

The mean plasma concentration-time profiles of the Test and Reference products are shown in Figure 2. Mean and their corresponding standard deviation (SD) of the parameters $AUC_{0-\infty}$, C_{max} , T_{max} and other associated pharmacokinetic parameters are shown in Table 2.

None of the effects analyzed by ANOVA for $AUC_{0-\infty}$ and C_{max} were statistically significant. There were no carryover effects for any of the parameters. Inter-subject variability was statistically significant for $AUC_{0-\infty}$ and C_{max} , while intra subject variability was not significant and it was similar for both formulations regarding these two parameters. The intra and inter subject residuals can be assumed normal and independent. Possible outlying observations was identified in one of the subjects who showed higher C_{max} for the Test formulation (118.260 ng/mL) than for the Reference formulation (61.416 ng/mL). These data were included in the statistical analysis.

The ratios between the Test and Reference products of $\ln AUC_{0-\infty}$ and $\ln C_{max}$ means and the 90% confidence intervals, corresponding to each parameter are shown in Table 3.

Discussion

To assure the quality of the formulations, before the Bioequivalence

study, the Test and Reference products were analyzed to determine their pharmaceutical equivalence. Both products met the requirements (Table 1) and the differences found in potency and dissolution tests were small, indicating that probably the drug would have similar pharmacokinetic parameters in both products.

There were no adverse reactions that required withdrawal of volunteers. All the adverse effects registered were considered mild and were resolved spontaneously at the end of each study period. The most frequently adverse effect observed in both treatments was bitter taste, as found in other study [18]. All events were consistent with side effects reported for Zopiclone.

In the analytical phase, 9 subjects showed Zopiclone plasma concentrations above the limit of quantification (5 ng/mL) from 0.33 to 24 hours, while in the remaining subjects these concentrations were

Parameter	Test product	Reference product
C_{max} (ng/mL)	72.815 (20.54)	74.315 (18.04)
T_{max} (h)	0.939 (0.39)	1.068 (0.69)
k_e (h ⁻¹)	-0.1270 (0.05)	-0.1318 (0.05)
$t_{1/2}$ (h)	6.377 (2.60)	6.064 (2.29)
AUC_{0-t} (ng*h/mL)	467.297 (92.21)	460.996 (115.81)
$AUC_{0-\infty}$ (ng*h/mL)	560.298 (118.58)	543.549 (136.97)

Table 2: Pharmacokinetic parameters (mean (SD)) of Zopiclone following a single 7.5 mg oral dose administration of the two products to healthy Colombian volunteers (n=26).

Parameter	Ratio Test/Reference	90% CI
C_{max} (ng/mL)	0.9932	89.97% - 104.84%
$AUC_{0-\infty}$ (ng/mL)*h	1.0059	97.38% - 110.59%

Table 3: Mean ratios for the Test product/ Reference product and the 90% confidence intervals of \ln -transformed data for C_{max} and $AUC_{0-\infty}$.

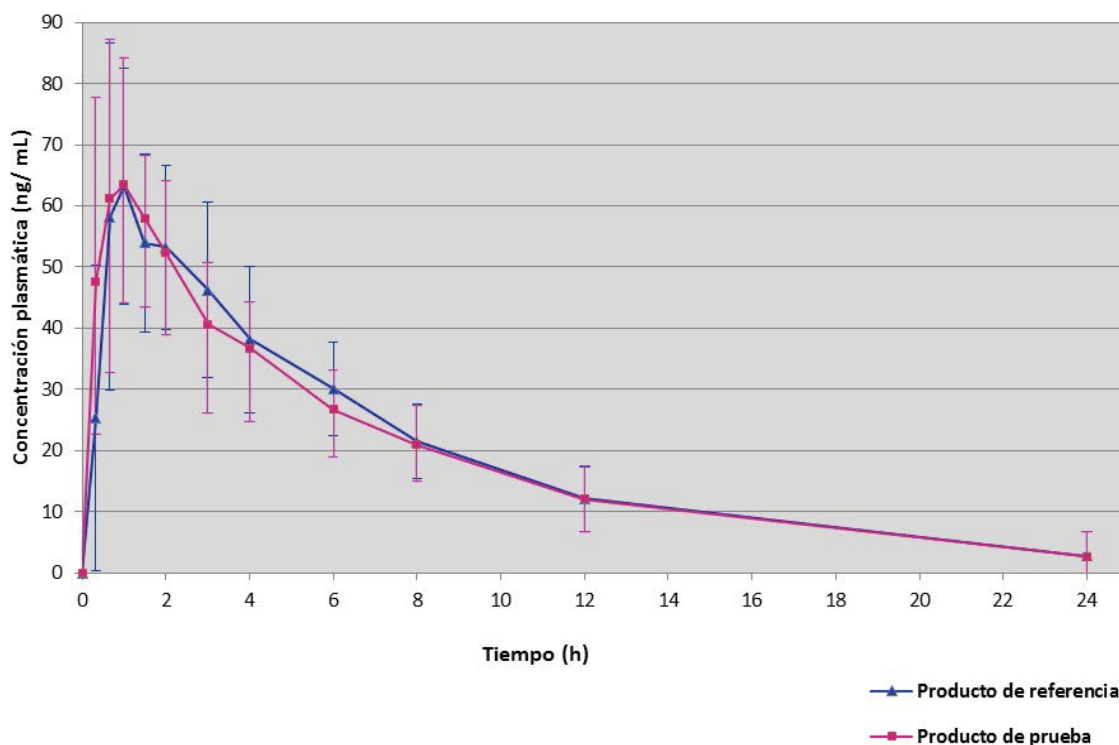


Figure 2: Mean (SD) plasma concentration-time profiles of Zopiclone 7.5 mg following a single oral dose administration of Test and Reference products to 26 Colombian healthy volunteers.

Parameter	Population							
	Colombian ^a n=26		European ^b n=26		Chinese 1 ^c n=24		Chinese 2 ^d n=22	
	T ^e	R ^f	T	R	T	R	T	R
AUC _{0-∞} (ng* <i>h</i> /mL)	467.297 ± 92.21	460.996 ± 115.81	460.319 ± 122.14	468.396 ± 165.36				
C _{max} (ng/mL)	72.815 ± 20.54	74.315 ± 18.04	67.869 ± 12.05	62.669 ± 18.27	72.68 ± 11.59	77.98 ± 15.06	20.26 ± 4.88	20.22 ± 4.59
T _{max} (h)	0.939 ± 0.39	1.068 ± 0.69	1.229 ± 0.86	1.794 ± 0.91	1.45 ± 0.64	1.33 ± 0.34	0.89 ± 0.31	1.03 ± 0.33
t _{1/2} (h)	6.377 ± 2.60	6.064 ± 2.29	6.572 ± 1.06 ^g	6.731 ± 1.72 ^g	5.40 ± 1.72	5.62 ± 1.95	6.58 ± 2.10	6.99 ± 2.65

^adata presented in this study; ^bReference [5]; ^cReference [20]; ^dReference [19], ^eTest product; ^fReference product; ^gcalculated with the coefficient of variation and the mean data reported in the document.

Table 4: Comparison of pharmacokinetic parameters (mean ± SD) found in several studies performed in different populations, following administration of Zopiclone 7.5 mg to healthy volunteers.

found from 0.33 to 12 hours. This event occurred in the same subjects, with both products and it may explain why the inter-subject variability was significant in this study. It could be interesting to consider these differences when subjects are recruited for the Bioequivalence trial for this drug.

Both formulations showed large variation in Zopiclone absorption, since in some subjects C_{max} was reached at the first sampling time, while in others was reached three hours after the drug administration. These differences were supported by high standard deviations obtained from the T_{max} data (Table 2) and were also found in other study [5].

Pharmacokinetic parameters obtained in this study such as C_{max}, T_{max} and t_{1/2} were similar to those found in other Bioequivalence trials [5, 19, 20] (Table 3), with the exception of C_{max} values reported by SI Tian-Mei et al. [19]. In that study, performed in 22 male volunteers in China, C_{max} values were very low for both Test (20.26 ± 4.88 ng/mL) and Reference product (20.22 ± 4.59 ng/mL) and were outside the range constructed with the other reported values (62.669 ± 18.27 ng/mL and 77.98 ± 15.06 ng/mL). The comparison of AUC_{0-∞} values was only possible with the data reported in reference [16], and it can be seen from Table 4 that they are quite close.

Because the cross over design removes the inter-subjects variability from the comparison between products, the significant difference found for this source or variation does not prevent bioequivalence assessment. The posteriori calculated power of the study, according to the formula proposed by Julious [21] gave a value of 99%. This result indicated that the sample size was adequate to conclude bioequivalence between these two formulations.

On the other hand, the exploratory data analysis identified a possible outlying subject with large differences in C_{max} between the Test and Reference products but not for AUC_{0-∞}. This inconsistency may be explained by an apparent controlled release of Zopiclone from the Reference product (T_{max} 90 minutes) in relation to the Test product (T_{max} 20 minutes) observed in this subject. When the dissolution rate of the drug from the dosage form is reduced, the drug is slowly absorbed and some pharmacokinetic parameters change: T_{max} increase, C_{max} decrease and AUC_{0-∞}, in theory, is the same. These differences are not extreme enough to affect the bioequivalence claim.

The statistical analysis showed similarity in relation to AUC_{0-∞} and C_{max} between the Test and Reference formulations. The 90% confidence intervals (Table 3) for the mean ratio (Test/Reference) fell within the 80%-125% interval established as bioequivalence criterion [17].

The results obtained indicate that the tested ZOPICLONE formulation (tablets of 7.5 mg) manufactured by Tecnoquímicas S.A. as Zopiclona MK[®] and Zopicloteg TG[®] is bioequivalent to the reference formulation, IMOVAME[®] 7.5 mg, manufactured by Aventis Farmacéutica Ltda. in terms of rate and amount of absorption,

according to the guidance of Instituto Nacional de Vigilancia de Medicamentos y Alimentos (INVIMA) [22] and FDA [23].

Acknowledgment

We thank toxicologist physicians Lina María Peña and Marie Claire Berrouet, Msc Andrés Mauricio Cogollo, Master student Ana Carolina Cáceres Molano and the undergraduate students Víctor Hugo Cuervo and Wbeimar León for their collaboration in the clinical phase of the study. We also thank Professor William Albeiro Álvarez by performing statistical analysis. This study was sponsored by Tecnoquímicas S.A.

References

1. <http://products.sanofi.ca/en/imovane.pdf>
2. www.who.int/medicines/areas/quality_safety/4.6_Zopiclone_Crit_Review.pdf.
3. Blanchard JC, Boireau A, Garret C, Julou L (1979) In vitro and in vivo inhibition by zopiclone of benzodiazepine binding to rodent brain receptors. *Life Sci* 24: 2417-2420.
4. Fernandez C, Martin C, Gimenez F, Farinotti R (1995) Clinical pharmacokinetics of zopiclone. *Clin Pharmacokinet* 29: 431-441.
5. <http://www.mhra.gov.uk/home/groups/lunit1/documents/websitesresources/con2023057.pdf>. Medicines and Healthcare products Regulatory Agency (MHRA). Zopiclone 7.5mg Tablets (Zopiclone) PL 08215/0049.
6. Bauer U (2001) Benzodiazepines and benzodiazepine receptor agonists in the treatment of sleep disorders—importance and dangers. Evaluation from the clinical medical view. *Z Arztl Fortbild Qualitatssich* 95: 17-21.
7. (2014) Metropolitan Life Desirable weights of adults.
8. World Medical Association-WMA (2013) Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects.
9. Ministerio de la Protección Social (2008) Buenas Prácticas Clínicas para Instituciones que conducen investigación con medicamentos en seres humanos. Resolución N° 2378.
10. Ministerio de la Protección Social (1993) Normas científicas, técnicas y administrativas para la investigación en salud. Resolución N° 8430.
11. Paw B, Misztal G (2000) Rapid and Simple Method for the Determination of Zopiclone in Plasma by HPLC. *J Liq Chrom and Rel Technol* 23: 2873-2882.
12. Nirogi RV, Kandikere VN and Mudigonda K (2006) Quantitation of zopiclone and desmethylzopiclone in human plasma by high-performance liquid chromatography using fluorescence detection. *Biomed Chromatogr* 20: 794-799.
13. Food and Drug Administration (2013) Guidance for Industry. Bioanalytical Method Validation.
14. Chow SC, Liu JP (2009) Design and analysis of bioavailability and bioequivalence studies. (3rd Edn) Chapman and Hall/CRC, Boca Raton.
15. The United States Pharmacopeial Convention. USP 32, NF 27 (2009). The United States Pharmacopeial Convention, Rockville 1: 557-560.
16. Schuirmann DJ (1987) A comparison of the two-one side test procedure and the power approach for assessing the equivalence of average bioavailability. *J Pharmacokinet Biopharm* 15: 657-680.
17. Food and Drug Administration (2001) Guidance for industry. Statistical Approaches to Establishing Bioequivalence.
18. Caille G, du Souich P, Spenard J, Lacasse Y, Vezina M (1984) Pharmacokinetic

- and clinical parameters of zopiclone and trimipramine when administered simultaneously to volunteers. *Biopharm Drug Dispos* 5: 117-125.
19. Si Tian-mei, Shu Liang, Zhang Hong-yan, Sun Li-li (2006) Bioequivalence of zopiclone tablet in Chinese healthy volunteers. *The Chinese Journal of Clinical Pharmacology* 2006-03.
20. Mengcai Z, Songwei P (2010) Bioequivalence of Zopiclone Tablet in Healthy Volunteers. *China Pharmacist* 2010-2013.
21. Julious SA (2004) Sample sizes for clinical trials with normal data. *Stat Med* 23: 1921-1986.
22. Instituto Nacional de Vigilancia de Medicamentos y Alimentos - INVIMA (1997) Guía de Biodisponibilidad y Bioequivalencia de Medicamentos. Acta 51, Sala Especializada de Medicamentos 15.
23. Food and Drug Administration (2003) Guidance for industry, bioavailability and bioequivalence studies for orally administered drug products.