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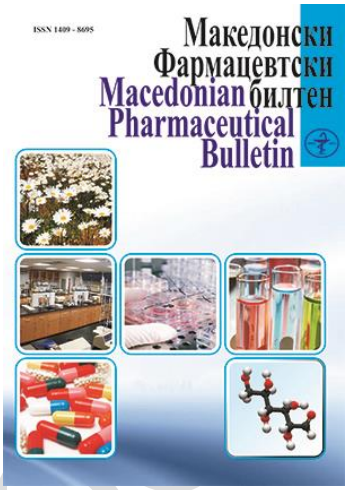

Title: **Comparative single-dose bioavailability study of two 10 mg Zolpidem tablet formulations in healthy volunteers**

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Comparative single-dose bioavailability study of two 10 mg Zolpidem tablet formulations in healthy volunteers

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Abstract

Zolpidem is a hypnotic agent with a chemical structure unrelated to benzodiazepines, barbiturates, pyrrolopyrazines, pyrazolopyrimidines or other drugs with known hypnotic properties. Zolpidem as conventional tablets is used as a hypnotic agent in the short-term management of insomnia, generally for periods not exceeding 7–10 days in duration.

The objective of this study was to evaluate and compare the relative bioavailability, and therefore the bioequivalence of Zolpidem 10 mg test formulation versus a reference Zolpidem 10 mg formulation, following a single dose administration under fasting conditions

The study was a single center, open, single dose, randomized, two - way crossover study in healthy male volunteers with a wash - out period of one week between study periods. Twenty-eight male healthy volunteers, aged 20-49 years were included into study. Blood samples for determination of zolpidem plasma concentrations were withdraw at 0 (pre-drug administration), 0.33, 0.66, 1, 1.33, 1.66, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 16 and 24 hours post-drug.

The zolpidem concentrations in plasma were determined with HPLC, using fluorescence detection.

The test formulation of zolpidem, dosed at 10 mg is bioequivalent for primary zolpidem parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) to the reference formulation after a single oral administration of 10 mg zolpidem. Both medications are well tolerated with no serious adverse events. Thus, in view of the clinical use, both formulations are exchangeable without restrictions.

Keywords: Zolpidem, bioavailability, bioequivalence study, single-dose

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Introduction

Zolpidem is an imidazopyridine-derivative sedative and hypnotic. Although zolpidem is structurally unrelated to the benzodiazepines, it shares some of the pharmacologic properties of benzodiazepines and has been shown to interact with the CNS γ-aminobutyric acid ($GABA_A$)-receptor-chloride ionophore complex at benzodiazepine (BZ) receptors. Unlike some benzodiazepines, which nonselectively activate central type 1 (BZ_1) and 2 (BZ_2) receptors, as well as peripheral type 3 (BZ_3) receptors, resulting in nonspecific pharmacologic actions, zolpidem reportedly may bind preferentially to BZ_1 receptors with a high affinity ratio of the α_1/α_5 subunits (Holm and Goa, 2000; Shirakawa, 2002; Swainston, 2005).

Zolpidem tartrate, has been in widespread clinical use for many years as a short-term treatment for insomnia. The main side-effects reported are GI upset, dizziness, headache and drowsiness (Foda and Ali, 2012; Greenbaltt and Roth, 2012).

Zolpidem has both a rapid absorption and onset of hypnotic effect. Bioavailability is 70% following oral administration. It demonstrates linear kinetics in the therapeutic dose range. The therapeutic plasma level is between 80 and 200 ng/mL. Peak plasma concentration is reached at between 0.5 and 3 hours after administration.

The distribution volume in adults is 0.54 L/kg and decreases to 0.34 L/kg in the elderly. Protein binding amounts to 92%. First pass metabolism by the liver amounts to approximately 35%. Repeated administration has been shown not to modify protein binding indicating a lack of competition between zolpidem and its metabolites for binding sites. The elimination half-life is short, with a mean of 2.4 hours and a duration of action up to 6 hours (Drover, 2004; Foda and Ali, 2012).

Study objective

The objective of this study was to evaluate and compare the relative bioavailability, and therefore the bioequivalence of tablets Lunata® 10 mg Alkaloid AD (test formulation) with

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Stilnox® (Synthelabo) 10 mg using a randomized two-way cross-over study in 28 healthy male volunteers after single oral dose under fasting conditions

Materials and methods

Experimental design of the study

The study was a single center, open, single dose, randomized, balanced, two - way crossover study in 28 healthy male volunteers with a wash-out period of one week between study periods.

Selection of study population

Twenty-eight male healthy volunteers, aged 20-49 years, with ideal body weight according to the Body Mass Index 18-30, non-smokers, were included into study. The volunteers' health condition was established on the base of history, physical examination, biochemical and hematological tests.

The study was started after the Ethical Committee for Medical Investigations and the Bureau for Medicines, Ministry of Health, Republic of Macedonia, had given their approval in writing.

A sample of 28 was estimated to be sufficient for the bioequivalence assessment of the investigated zolpidem under fasting conditions. The sample size was estimated using nQuery software based on the data from pilot study and determined intra-subject variability when drug is administered under fasting conditions. Under such conditions intra-subject variability should not exceed 20%. With intra-subject coefficient of variation of 20% and a test/reference ratio within 0.95-1.05, the study should have a power of at least 80% to show bioequivalence with 24 subjects (Diletti et al., 1991).

Prior to entering the study, the volunteers were informed about the administered preparations and the possible risk for their health. All of them signed the "Informed consent".

Inclusion criteria

The subjects with the following criteria were eligible for this trial:

- Male subjects aged between 18-55 years, Caucasian race,

- Body Mass Index (weight/height^2) in the range 18-30 kg/m^2,
- Non-smokers,
- Clinically normal vital signs,
- Clinically normal medical history,
- Clinically normal findings on physical examination,
- Clinically normal findings for hematology and clinical chemistry of blood and urine,
- No history of alcohol and/or drug abuse,
- Able to communicate and co-operate with the investigator and his staff,
- HBsAG, AntiHCV and AntiHIV tests, taken before of study start, must be negative,
- Able to give written informed consent.

The above listed analyses were performed within 14 days of the study start.

Exclusion criteria

Subjects meeting one or more of the following criteria were not selected:

- Chronic illness,
- Any clinically significant illness during 4 weeks before the study (i.e. before the first dosing),
- Clinically significant abnormalities of medical history, on physical examination, of hematology, biochemistry and urinalysis results and of the ECG,
- Positive screen for HBsAg, anti-HCV, anti-HIV-1/HIV-2,
- Use of vitamins or herbal products within 2 weeks before the study,
- Use of any other over-the-counter medication or prescription medication within 4 weeks before the study,
- Clinically significant history of reaction to drugs in the past,
- Participation in other clinical studies and/or blood donation within 2 months before the study,
- Inability or unwillingness to comply with the provisions of this protocol.

Drug information

In the study, as a test drug (A), Lunata® 10 mg in form of tablet, product of Alkaloid AD was used. Reference drug (B) was Stilnox® 10 mg in form of tablet, product of Synthelabo. The formulations were administered in fasting conditions. Drugs were administered orally in the form of tablets with 240 mL of water at ambient temperature. The volunteers received the tested formulations according the randomization scheme. After washout period of 7 days the other drug was administered.

Sample collection and drug concentration measurement

Blood samples for determination of zolpidem plasma concentrations were withdraw at 0, (pre-drug administration), 0.33, 0.66, 1, 1.33, 1.66, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 16. and 24 hours post-drug (18 blood samples).

The zolpidem concentrations in plasma were determined with High Performance Liquid Chromatography, using fluorescence detection. The method was specific for zolpidem since no interfering peaks are appearing in the chromatogram at the retention time of zolpidem. The method had linear response for the concentration levels from 2 to 300 ng/mL zolpidem. The lower limit of quantification for zolpidem was 2 ng/mL (Zendelovska et al. 2015).

Bioequivalence/Bioavailability parameters

According to the obtained plasma concentrations/time data of zolpidem the following pharmacokinetic parameters were calculated using software KINETICA™ 4.2 (Innaphase corporation, USA).

Primary parameters: AUC_{0-t} and $AUC_{0-\infty}$ (area under the curve of the plasma concentrations until the last sampling time and infinity), C_{max} (maximum plasma concentration).

Secondary variable: t_{max} (time of reaching the maximum plasma concentrations).

Statistical analysis

The correspondent 90% confidence intervals for $AUC_{0-\infty}$, AUC_{0-t} and C_{max} of the tested preparation as a ratio to the correspondent values of the referent preparation using parametric and nonparametric methods without or with log-transformation of data were calculated. The

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differences in t_{max} of test and reference preparations were analysed by means of a non-parametric analysis of variance at a 90% confidence interval.

Results and discussion

Thirty (30) male Caucasian subjects were recruited for participation in the trial. Among the 30 subjects recruited for the trial, twenty-eight (28) were included in the study. There was no drop out.

Demographic data from the study shows that participants have mean ± SD age (32.321±9.553), weight (84.786±7.500), height (178.464±6.780), and body mass index (26.607±1.257).

The maximum plasma concentrations of zolpidem (155.186±48.82 μg/L) and (157.592±53.97 μg/L) was attained in about 0.805 ± 0.476 hours and 0.8525 ± 0.449 for both test and reference, respectively. Total area under the curve (AUC) mean ± SD values were 574.7728±340.4059, and 537.9394±290.3884 μg/L x h for both test and reference tablets.

The primary and secondary pharmacokinetic parameters (mean ± SD) are presented in Tables 1 and 2 for test and reference formulations.

Table 1

Table 2

Figures 1 and 2 illustrate the mean plasma concentration time-course of zolpidem obtained after the administration of 10 mg zolpidem as treatment A (Test) and treatment B (Reference) in the twenty-eight healthy young male volunteers, in linear and semi-logarithmic scale.

Fig. 1

Fig. 2

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Statistical analysis

The results of the statistical analysis of the pharmacokinetic parameters of zolpidem between the test and reference formulations are resumed in the Table 3.

Table 3

Safety

During the period I of the study, one (1) volunteer had transitory adverse reaction (nausea) which resolved approximately 1-2 hours after drug administration without medication. Other 17 volunteers had transitory adverse reactions (somnolence/or dizziness) which resolved spontaneously approximately 30 minutes to 2 hours after drug administration. During the period II 18 volunteers had transitory adverse reactions (somnolence/or dizziness) which resolved spontaneously approximately 30 minutes to 2 hours after drug administration

The study was carried out according to the protocol. All the pharmacokinetic and safety assessments were performed as planned in the protocol. All clinical work was conducted in compliance with Good Clinical Practices (GCP) as referenced in the ICH guidelines and with the directive *2001/20/EC* of the European Parliament (Directive 2001/20/EC, 2001; EMEA/CPMP/ICH/135/95.

Analysis of pharmacokinetic parameters

From the 28 subjects included in this study, 28 were analyzed and included in the pharmacokinetic and statistical analysis for the zolpidem.

After the administration of 10 mg zolpidem as test and reference formulation the mean plasma concentration time-courses of zolpidem present the same pharmacokinetic profiles with minor differences between the two formulations.

The peak plasma concentration is bioequivalent between the Test formulation and the Reference formulation (respectively 155.1857±49.81585 μg/L and 157.5918±53.96815 μg/L). The peak plasma concentration of zolpidem is attained at about 0.805±0.476029 hours for the test and 0.8525±0.44907 hours for reference formulations. The AUC parameters showed that the AUC_{0-t} of zolpidem (556.2515±326.6312 μg/L x h and 524.7117±283.7047 μg/L x h respectively for the

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test formulation and the reference formulation) and the $AUC_{0-\infty}$ of zolpidem (574.7728±340.4059 μg/L x h and 537.9394±290.3884 μg/L x h respectively for the test formulation and the reference formulation) are bioequivalent after the administration of the test formulation and after the administration of the reference formulation.

The statistical analysis of the half-life of elimination, clearance, rate of elimination and mean residence time showed no significant difference between the values of test and reference formulation.

Safety analysis

All the subjects included in this study (28 subjects) were considered for the safety analysis. Both treatments (Treatment A and Treatment B) appear to be safe and well tolerated after single oral dose of Zolpidem 10 mg tablet to healthy male volunteers under fasting conditions. No death, no serious adverse event or adverse event did occur during the study.

The safety analysis shows that the treatments were well tolerated. In each group there were eighteen adverse events in both study period. Adverse effects were transitory and graded from mild (15 volunteers with test and 14 volunteers with reference formulation) to moderate (3 volunteers with test and 4 volunteers with reference formulation).

From cardiovascular safety point of view, the cardiovascular data, blood pressures, heart rates and electrocardiogram parameters, didn't show clinically significant changes for all the subjects.

Conclusion

As conclusion, the test formulation dosed at 10 mg is bioequivalent for primary zolpidem parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ parameters) to the reference formulation after a single oral administration of 10 mg zolpidem. The peak plasma concentration of zolpidem (C_{max}) is approximately equal in both formulations and the AUC parameters are bioequivalent to the reference formulation.

Both medications are well tolerated with no serious adverse events. Thus, in view of the clinical use, both formulations are exchangeable without restrictions.

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Резиме

Компаративна студија на биорасположивост на две формулации на Золпидем во форма на таблети од 10 mg по еднократна апликација кај здрави доброволци

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Клучни зборови: Золпидем, биорасположивост, биоеквивалентна студија, еднократна доза

Золпидем е хипнотички лек кој по својата структура се разликува од бензодиазепините, барбитуратите, пиролопиразините, пиразолопиримидините или другите лекови со познати хипнотички карактеристики. Золпидем во форма на конвенционални таблети се користи како хипнотички лек за краткотраен третман на инсомнија, обично за период не подолг од 7-10 дена.

Целта на оваа студија беше да се евалуацира и спореди биорасположивоста, а потоа и биоеквивалентноста на Золпидем тест формулација od 10 mg во однос на референтна формулација на золпидем од 10 mg по нивна еднократна администрација на гладно.

Студијата беше спроведена како моноцентрична, отворена, со еднократна апликација, рандомизирана, двојно вкрстена студија кај здрави машки доброволци со "wash-out" период од две недели помеѓу двата периоди. Дваесет и осум машки здрави доброволци на возраст од 20-49 години беа вклучени во студијата. Примероците на крв за одредување на плазматските концентрации на Золпидем беа земани во нултото време (пред администрација на лекот),како и по 0,33; 0,66; 1; 1,33; 1,66; 2; 2,5; 3; 3,5; 4; 5; 6; 8; 10; 12; 16

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и 24 часа по администрација на лекот. Плазматските концентрации на золпидем беа одредувани со HPLC, со користење на флуоресцентна детекција.

Испитуваната формулација на Золпидем во доза од 10 mg е биоеквивалентна во однос на примарните параметри на Золпидем (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) во однос на референтната формулација по еднократна орална администрација на Золпидем во доза од 10 mg. Двата лека се добро толерирани, без посериозни несакани дејства. Оттука, од аспект на клиничка употреба, двете формулации се заменливи без ограничувања.

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Table 1. Mean pharmacokinetic parameters of zolpidem (Test)

Preparation		ZOLPIDEM (TEST)				
Parameter	n	Mean	Median	Min	Max	SD
$AUC_{0-\infty}$ (µg/L x h)	28	574.7728	462.119	127.176	1384.39	340.4059
AUC_{0-t} (µg/L x h)	28	556.2515	450.4145	119.483	1293.92	326.6312
C_{max} (µg/L)	28	155.1857	152.23	55.23	260.91	49.81585
t_{max} (h)	28	0.805	0.66	0.33	2	0.476029

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Table 2. Mean pharmacokinetic parameters of zolpidem (Reference)

Preparation	ZOLPIDEM (REFERENCE)				
Parameter	N	Mean	Median	Min	Max	SD
$AUC_{0-\infty}$ (µg/L x h)	28	537.9394	508.933	174.846	1248.78	290.3884
AUC_{0-t} (µg/L x h)	28	524.7117	494.8085	179.791	1219.61	283.7047
C_{max} (ng/ml)	28	157.5918	145.86	79.5	282.38	53.96815
t_{max} (h)	28	0.8525	0.66	0.33	2	0.44907

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Table 3. Statistical analysis of the pharmacokinetic parameters of zolpidem

Used test for the statistical comparison	t_{max}	C_{max}	AUC_{0-t}	$AUC_{0-\infty}$
Wilcoxon test	S	-	-	-
ANOVA				
Treatment		N.S. (p>0.05)	N.S. (p>0.05)	N.S. (p>0.05)
Subject		N.S. (p>0.05)	N.S. (p>0.05)	N.S. (p>0.05)
Period		N.S. (p>0.05)	N.S. (p>0.05)	N.S. (p>0.05)
Bioequivalence test				
90% standard confidence interval		0.8851-1.0947	0.9359-1.1183	0.9432-1.125
Two one-sided T-tests (Schuirmann)		can conclude equivalence	can conclude equivalence	can conclude equivalence

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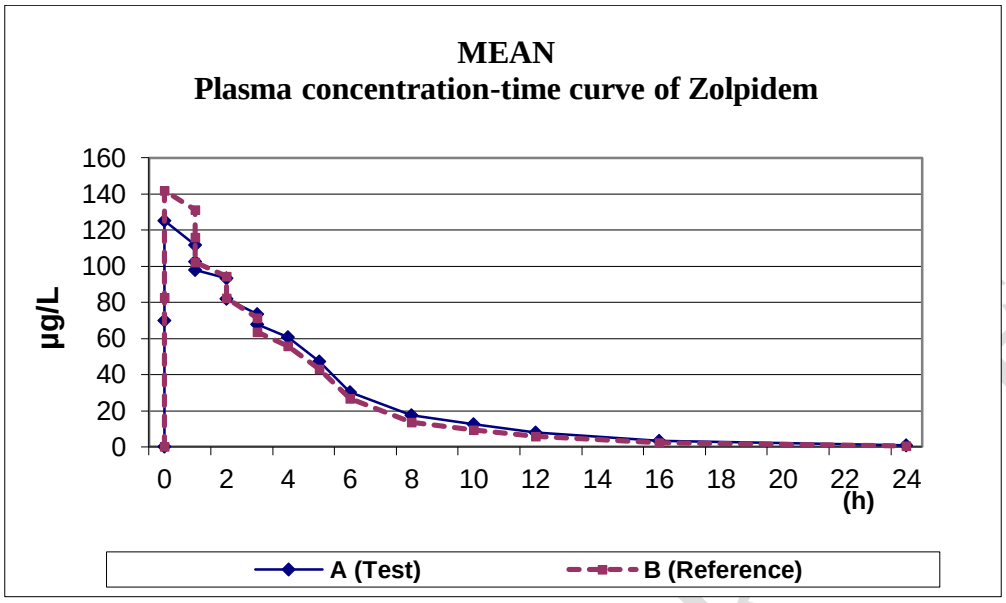

Fig. 1. Mean plasma concentration-time curve of zolpidem after single administration of test and reference formulation of 10 mg zolpidem.

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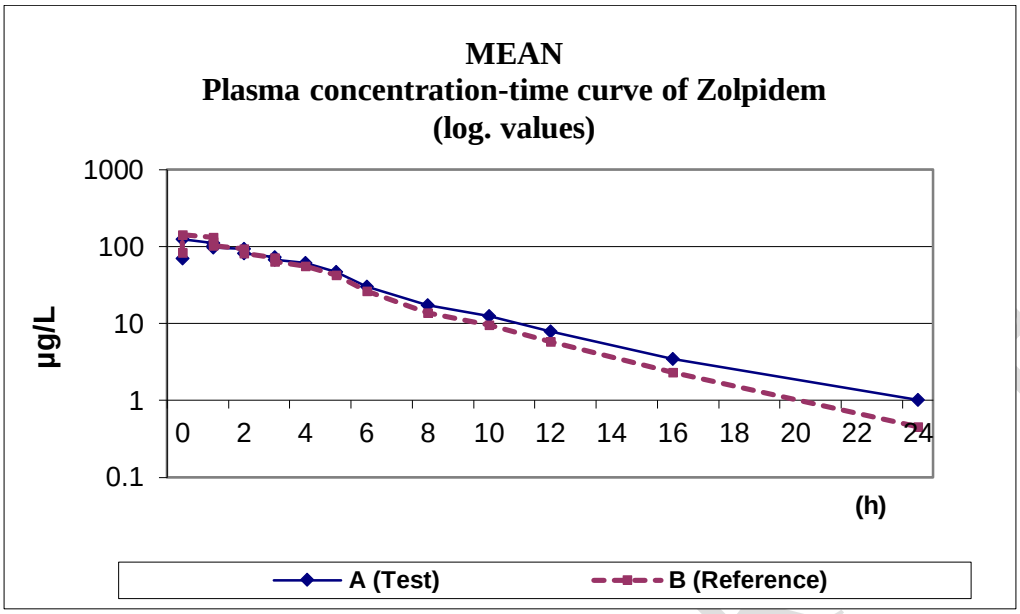

Fig. 2. Mean plasma concentration-rime curve of zolpiden (log. values) after single administration of test and reference formulation of 10 mg.

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