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BUMETANIDE PHARMACOKINETICS - PHARMACOKINETICS BIOAVAILABILITY AND BIOEQUIVALENCE EVALUATION OF TWO BRANDS OF BUMETANIDE 1MG TABLETS IN HEALTHY SUBJECTS

FARMAKOKINETIKA BUMETANIDA - FARMAKOKINETIKA, BIOLOŠKA RASPOLOŽIVOST I BIOEKVIVALENCIJA DVE FORMULACIJE TABLETA OD 1 MG BUMETANIDA KOD ZDRAVIH DOBROVOLJACA

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Key words
bioequivalence; bumetanide; HPLC; volunteers

Ključne reči
bioekvivalencija, bumetanid, HPLC, dobrovoljci

Abstract

This paper describes a bioequivalence study with two oral bumetanide (1 mg) tablets formulations. The reference (R) preparation was Yurinex/Leo, Kopenhagen, and the test (T) preparation was Bumetanide/Galenika, Belgrade, Serbia. The aim of this study was to investigate the relative bioavailability of 1 mg new tablets and pharmacokinetics of bumetanide. The study design was open, randomized, two-period, two-sequence, two-treatment with crossover involving 18 healthy male subjects. All subjects completed the study. Bumetanide plasma concentrations were measured utilizing a sensitive, reproducible and accurate HPLC method. Pharmacokinetic parameters used to assess bioequivalence were $AUC_{0\text{-last}}$, $AUC_{0\text{-inf}}$ for the extent of absorption and C_{max} and tmax for the rate of absorption. Statistical evaluation of $AUC_{0\text{-last}}$, $AUC_{0\text{-inf}}$ and C_{max} was done after semilogarithmic transformation using a two-way analysis of variance (ANOVA). t_{max} values were tested using the distribution-free Hodges-Lehman interval. The parametric 90% confidence intervals for ratio T/R ranged from 96.50 – 112.30% (point estimate 104.42%) for $AUC_{0\text{-last}}$, 96.60 – 113.20% (point estimate 104.22%) for $AUC_{0\text{-inf}}$ and 97.20 – 108.52% (point estimate 101.36%) for C_{max} respectively. Based on the results of AUC, C_{max}, t_{max}, K_{el} and $t_{1/2}$, there were no statistically significant differences and the two bumetanide preparations are equivalent with respect to rate and extent of absorption.

INTRODUCTION

Bumetanide (3-n-butylamino-4-phenoxy-5-sulphamylbenzoic acid) is a potent high ceiling loop diuretic with a potency 40 to 60 times greater than frusemide.

Loop diuretics are mainly used to relieve edema associated with congestive heart failure, hepatic cirrhosis and renal impairment diseases and to treat high blood pressure. However, diuretics may also be used by athletes as masking agents to decrease weight. Taken as masking agents, diuretics increase urine production and decrease urinary concentrations of banned performance-enhancing agents, such as anabolic steroids [1].

Shaping the diuretic response to loop diuretics for effective use in edemateous patients is important for the successful treatment in these patients who are more resistant to loop diuretics than normal subjects [2-8].

Bumetanide has been shown to be absorbed from different segments of the gastrointestinal tract of rats [9]. The bioavailability of bumetanide in humans is not decreased, when co-administered with food [10].

A two-compartment model adequately fitted the intravenous data [11].

Some pharmacokinetic parameters of bumetanide were infusion time-dependent in rabbits [12] and it might be due to saturable metabolism of bumetanide.

In the literature, there have been a limited number of studies on the pharmacokinetics of bumetanide in either normal human subjects [10, 11, 13, 14] or in disease states [15]. This paper describes determination of the pharmacokinetics and bioequivalence of two tablet formulations of bumetanide in human volunteers. Bumetanide/Galenika, Belgrade, Serbia was used as the test and Yurinex/Leo, Kopenhagen as the reference product given orally under fasting conditions.

SUBJECTS, MATERIALS AND METHODS

Subjects

Healthy male non-smoker volunteers [18] from the local population were screened and enrolled in the trial. Before being admitted to the clinical study, each prospective candidate signed an informed consent form. The mean age of volunteers was 30.1 ± 7.6 years, mean height was 178.7 ± 8.6 cm and mean body weight was 68.1±12.9 kg. Clinical and laboratory examinations were performed within 14 days before day one of the study and not later then seven days after the second application. Standard laboratory screening included medical history, a complete clinical examination including vital signs and an electrocardiogram. Baseline laboratory tests were done: hematology and clinical chemistry of blood samples and urinalysis. The results of all laboratory tests, including vital safety test and an ECG, were available before the final inclusion of each subject.

The volunteers were also checked for the presence of HBsAg and HIV antibodies in serum. The post-study physical and laboratory examinations were similar to the initial entry examination with the exception of HIV-Ab and HBsAg determination.

The protocol was approved by an independent Ethics Committee. The study was conducted in compliance with the principles of the Declaration of Helsinki 1964 and its amendments (Tokyo 1975, Venice 1983, Hong Kong 1989). The two study periods were separated by a washout period of one week.

Study design and blood samples

The study was open, randomized, two-formulation, two-period, two-sequence, cross-over bioequivalence study of bumetanide following a single dose oral administration of the test formulation Bumetanide/Galenika 1 mg tablets and the reference formulation Yurinex/Leo, Kopenhagen 1 mg tablets, involving 18 healthy male volunteer subjects. The subjects were given sequential numbers (1-18) and then divided into two groups: the first group with odd numbers was given the Test (T) then Reference (R) whereas the second group with even code numbers was given the Reference (R) followed by the Test (T).

On the first day of each period of the study, a single dose (2 x 1 mg in each case) of either the test or reference was administered with 200 ml of bottled water according to the randomization. No intake of alcohol, caffeine or xanthine-containing food and drink, and no smoking was allowed within 72 hours of each dose.

The subjects fasted for at least ten hours (food) and three hours (drink) before receiving their morning breakfast at the study center.

Intake of alcohol, caffeine and xanthine-containing food and drink and smoking were prohibited during post-dose confinement period. Subjects received standardized meals approximately six hours after dosing during the confinement period. The subjects were not allowed to lie down or sleep during the 12-hour period after dosing. No strenous activity was allowed during the confinement periods. Blood samples were collected just before drug administration and at 0.25, 0.5, 0.75, 1, 1.33, 1.66, 2, 2.5, 3, 3.5, 4, 6, 8 hours after administration.

The blood samples were obtained from a short intravenous catheter (Abbocath) and collected into 12 ml tubes, using heparin as an anticoagulant. Blood samples were immediately centrifuged (3500 rpm, 10 min), and the separated plasma was transferred into 2.0 ml Eppendorf tubes and stored at -70 ºC until analysis.

HPLC analysis of plasma samples

Bumetanide concentrations were determined using a rapid high-pressure liquid chromatographic method which is specific for bumetanide [16].

Pharmacokinetic and statistical analysis

The bioequivalence was tested using the three primary parameters, $AUC_{0\text{-last}}$, $AUC_{0\text{-inf}}$ and C_{max}. C_{max} and the time of the maximum plasma concentration (t_{max}), which is also a parameter to test bioequivalence, were determined from the concentration-time data for each volunteer [17].

$AUC_{0\text{-last}}$ was calculated using the trapezoidal rule, whereas $AUC_{0\text{-inf}}$ was computed using the equation $AUC_{0\text{-inf}} = AUC_{0\text{-last}} + C_n/K_{el}$. Elimination half-life ($t_{1/2}$) was computed using the equation $t_{1/2} = \ln(2)/K_{el}$.

From the terminal log-decey phase, elimination rate constant (K_{el}) was estimated using linear regression. A bioexponential equation was fitted to the data by the least-square method for the determination of absorption rate constant (K_a).

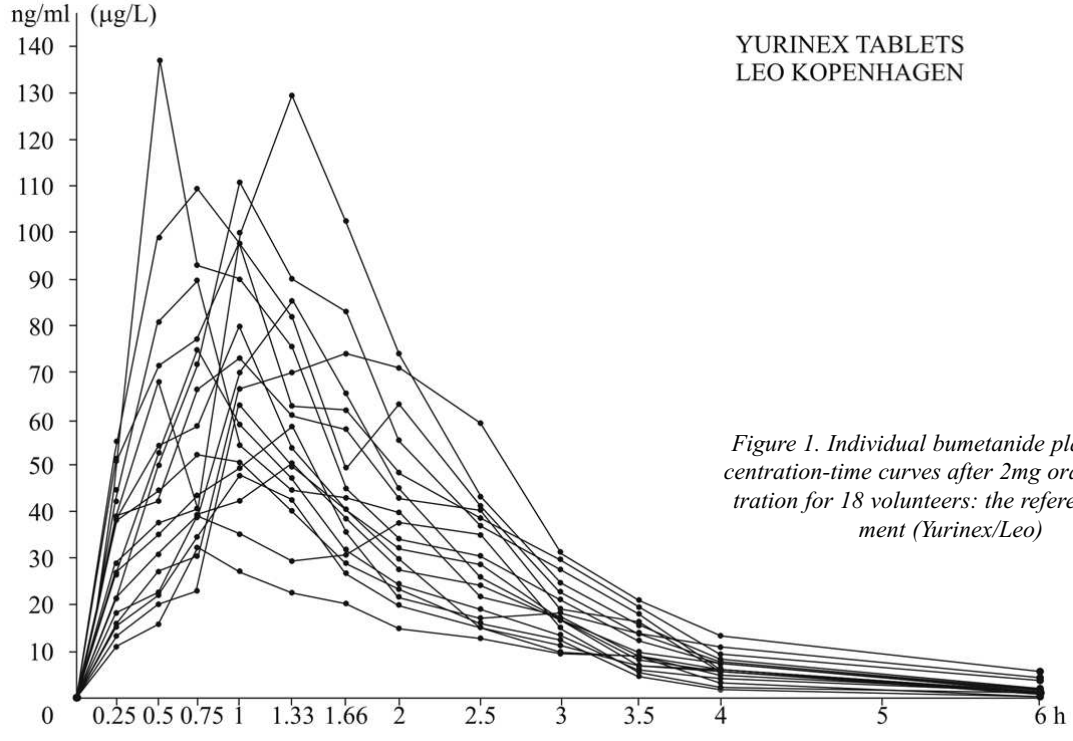

Figure 1. Individual bumetanide plasma concentration-time curves after 2mg oral administration for 18 volunteers: the reference treatment (Yurinex/Leo)

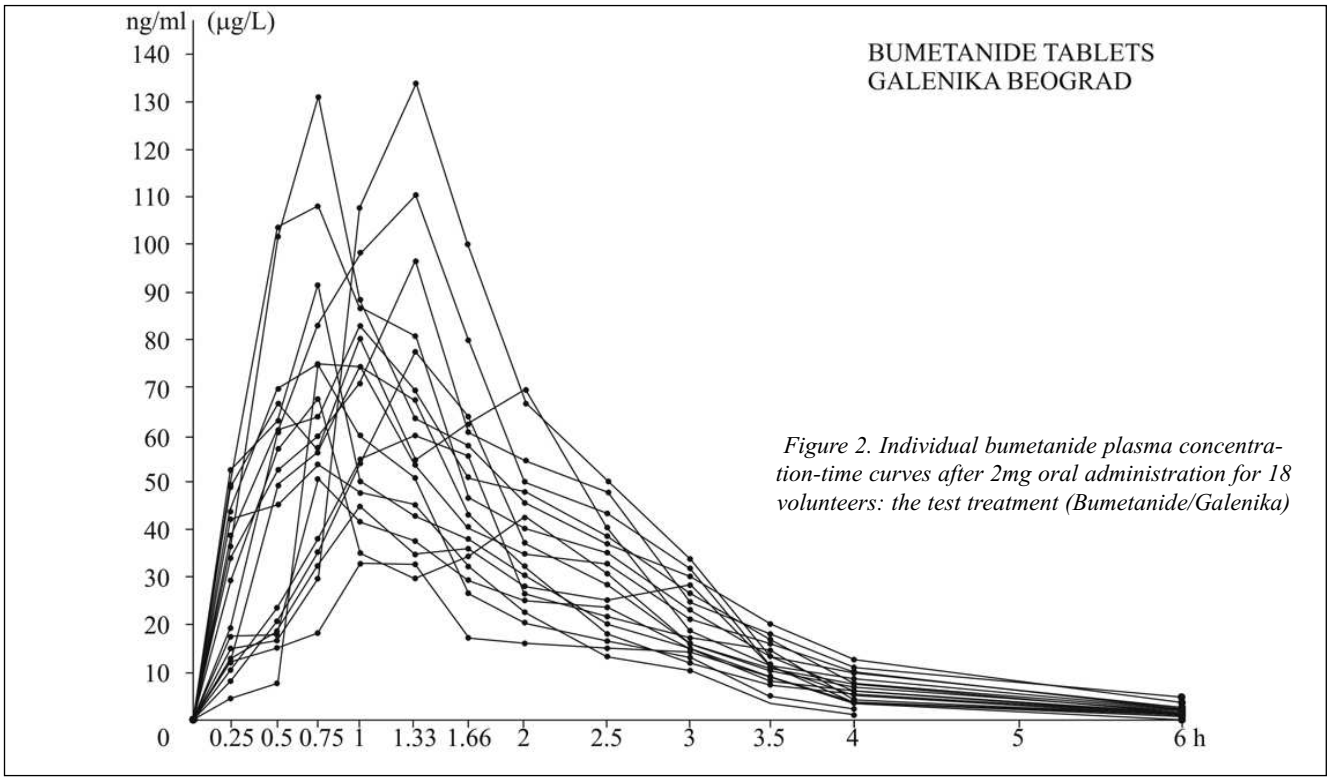

Figure 2. Individual bumetanide plasma concentration-time curves after 2mg oral administration for 18 volunteers: the test treatment (Bumetanide/Galenika)

For the parameters of $AUC_{0\text{-last}}$, $AUC_{0\text{-inf}}$ and C_{max} a multiplicate model was assumed, and an analysis of variance (ANOVA) for 2x2 cross-over design was applied using the respective Ln-transformed data. ANOVA was performed using the F test. The independent factors of the model were inter-subjects: sequence and subjects (within sequence) and intra-subjects: treatment and period. For estimation of bioequivalence the 90% confidence intervals (CIs) of the geometric mean ratio test/reference for $AUC_{0\text{-last}}$, $AUC_{0\text{-inf}}$ and C_{max} were calculated assuming a multiplicative model. The accepted bioequivalence range for these parameters was 80% and 125%. Comparison of tmax values was tested using the distribution-free Hodges-Lehman interval. For K_a only descriptive statistics are given.

Using power analysis [18] ($\beta = 0.2$) it was determined that the power of the analysis of variance (ANOVA) was > 0.8 at 90% CI, indicating that a total of 18 subjects would be sufficient for the purposes of study.

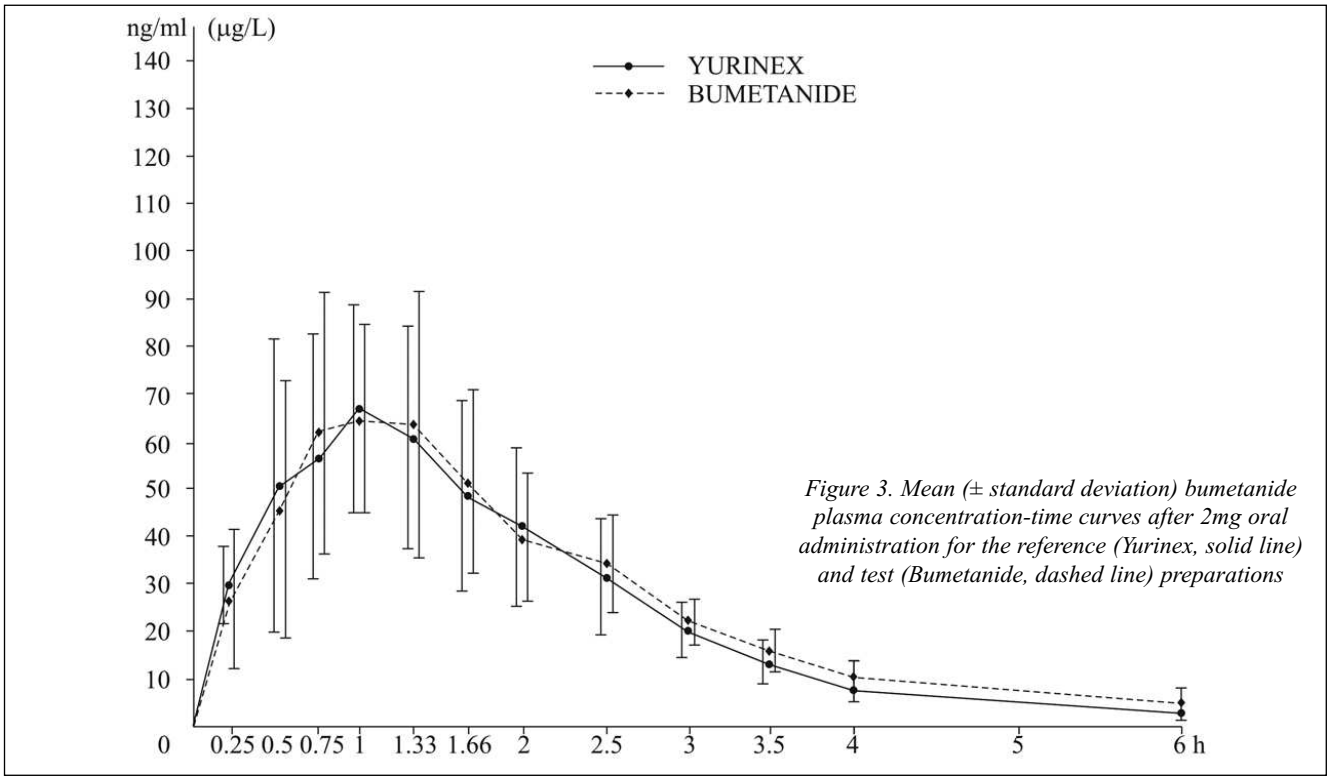

Figure 3. Mean (± standard deviation) bumetanide plasma concentration-time curves after 2mg oral administration for the reference (Yurinex, solid line) and test (Bumetanide, dashed line) preparations

Probability of exceeding the limits of acceptance (80% - 125%) was obtained by the two 1-sided t tests described by Schuirmann [19]. The formulations were considered bioequivalent if the Ln-transformed ratios of AUC and C_{max} were within the predetermined equivalence range of 80% to 125% and if P was ± 0.05 for the 90% CIs. All pharmacokinetic and statistical analyses were performed using WinNonlin version 5.1, (2008).

RESULTS

All 18 volunteers successfully completed the trial according to the protocol. No pathological changes were found during clinical screening before the beginning of the study and after the end of the trial. The clinical investigator found no abnormal laboratory values. Therefore, the study was carried out with no occurence of clinically significant problems. No moderate or serious adverse effects (AEs) were observed in either day of dosing. Any adverse events were classified as minor and mild, unlikely to be related to the study medication and disappeared complete without the need for medical intervention. The drug in both preparations was well-tolerated without any other symptoms or disturbances. The individual bumetanide plasma concentration-time curves for the reference and test preparations are graphically presented in Figures 1 and 2, respectively. The mean (± SD) bumetanide plasma concentration-time curves for the reference and test preparations are graphically presented in Figure 3.

Figure 3 shows that the mean plasma concentration profile of the two brands was closely similar. Peak concentrations of 66.93 ng/ml and 65.66 ng/ml for bumetanide were attained at 1 h after drug administration and these then declined and were detectable up to 6 h post dose.

Descriptive statistics of the pharmacokinetic parameters for bumetanide reference and test preparations are summarized in Table 1 which shows geometric mean, SD, for $AUC_{0\text{-last}}$, $AUC_{0\text{-inf}}$, C_{max}, t_{max}, K_a and $t_{1/2}$. For both drugs, the mean values of all parameters were very similar for the two formulations.

The results of the bioequivalence analysis are given in Table 2.

In the present study, analysis of variance (ANOVA) showed that there were no significant differences between the two preparations using the pharmacokinetic parameters $AUC_{0\text{-last}}$, $AUC_{0\text{-inf}}$, Cmax and $t_{1/2}$. The extent as well as the rate of absorption reflected by the $AUC_{0\text{-last}}$, $AUC_{0\text{-inf}}$ and Cmax respectively, indicate bioequivalence since they lie within required 90% confidence interval of 80 – 125 %.

Table 2 shows the 90% CIs of the ratios (test/ reference) for the ln-transformed values of $AUC_{0\text{-last}}$, and $AUC_{0\text{-inf}}$ (as an index of the extent of absorption), C_{max} (as an index of rate of absorption); the probability exceeding the limits of acceptance (Schuirmann's two 1-sided t tests); and the power of the test [19]. The 90% CIs for the corresponding ratios of $AUC_{0\text{-last}}$, $AUC_{0\text{-inf}}$ and C_{max},were within the 80% to 125% range. All P values were < 0.05. Similar results were found for data without a logarithmic transformation and for $t_{1/2}$ and tmax values.

DISCUSSION

The results of our study suggest that the reference-test formulations were not statistically different in terms of their pharmacokineic parameters (AUC and C_{max}) were found to be within the predetermined range (80% - 125%) and the Schuirmann's two one-sided t test procedure (probability of exceeding limits of acceptance) found all probability values < 0.05, the hypothesis that the estimated parameters exceeded limits of acceptance was rejected.

Pharmacokinetic parameters determined in our study are similar to previously reported values in other healthy volunteer ethnic groups [11, 13, 14].

No moderate or seriuos AEs were reported by the investigators. Potential recall bias of AEs in this study was not likely because only one dose of each formulation was administered during each treatment period, subjects were under medical surveillance in the clinical unit, and the duration of the washout period was only 7 days.

CONCLUSION

In conclusion, the two bumetanide preparations are equivalent with respect to the rate and extent of absorption and it can be assumed to be therapeutically equivalent and exchangeable in clinical practice.

Formulations were generally well tolerated.

Acknowledgement

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Table 1. Pharmacokinetic parameters for bumetanide Reference and Test preparations after oral dose with 2 mg bumetanide in 18 volunteers. Values are geometric mean ± standard deviation

Pharmacokinetic parameter	Reference drug (Yurinex^R)		Test drug (Bumetanide)	
	Mean	Standard Deviation	Mean	Standard Deviation
$AUC_{0\text{-last}}$ (h · ng/ml)	151.4	67.7	154.0	69.5
$AUC_{0\text{-inf}}$ (h · ng/ml)	153.9	77.9	156.5	73.4
C_{max} (ng/ml)	79.2	28.5	80.1	27.4
t_{max} (h)	0.98	0.31	0.98	0.25
K_a (h^{-1})	2.62	0.45	2.37	0.41
$t_{1/2}$ (h)	1.08	0.31	1.12	0.33

$AUC_{0\text{-last}}$ = area under the curve from the first until the last sampling
$AUC_{0\text{-inf}}$ = area under the curve extrapolated to infinity
C_{max} = maximum concentration
t_{max} = time of the maximum concentration
K_a = absorption rate constant
$t_{1/2}$ = elimination half-life

Table 2. Ratios, 90% confidence intervals of natural log-transformed data, the probability of exceeding the limits of acceptance (80%-125%), and power test results of 2 oral formulations of bumetanide after a single dose of 2 mg.

Pharmaco-kinetic parameter	Ratio % (Ref)	CI 90% Classical	CV ANOVA (%)	CI 90% Westlake	Criteria for accepting Bioequivalence	Two one-sided t test Schuirmann	P	Power of the analysis	Conclusion
$LnAUC_{0\text{-last}}$ (h · ng/ml)	104.42	96.50-112.30	29.44	97.02-117.03	(80%-125%)	P(q<80%) = 0.0000 P(q>125%) = 0.0000	P<0.05	0.9999	Bioequivalent
$LnAUC_{0\text{-inf}}$ (h · ng/ml)	104.22	96.60-113.20	28.37	97.23-114.10	(80%-125%)	P(q<80%) = 0.0000 P(q>125%) = 0.0000	P<0.05	0.9997	Bioequivalent
LnC_{max} (ng/ml)	101.36	97.20-108.52	29.51	94.54-107.35	(80%-125%)	P(q<80%) = 0.0000 P(q>125%) = 0.0000	P<0.05	0.9998	Bioequivalent
$t_{1/2}$ (h)	103.07	97.77-110.01	29.40	94.90-111.20	(80%-125%)	P(q<80%) = 0.0000 P(q>125%) = 0.0000	P<0.05	0.9999	Bioequivalent

CI = confidence interval

CV = coefficient of variation

Apstrakt

Ispitana je bioekvivalencija tableta bumetanida od 1 mg dva proizvođača. Referentni preparat (R) je Yurinex/Leo, Kopenhagen, a testirani (T) Bumetanid/Galenika, Beograd. Cilj studije je ispitati relativnu biološku raspoloživost novih tableta i farmakokinetiku bumetanida. Studija je otvorena, ukrštena, randomizovana, sa dva perioda, dve sekvence i dva tretmana, na 18 zdravih dobrovoljaca. Svi dobrovoljci su bez neželjenih dejstava završili studiju. Koncentracije bumetanida u plazmi su merene osetljivom HPLC metodom. Farmakokinetički parametri za utvrđivanje bioekvivalencije su bili $AUC_{0\text{-last}}$, $AUC_{0\text{-inf}}$ za količinu apsorbovanog leka i C_{max} i t_{max} za brzinu apsorpcije. Statistička obrada AUC i C_{max} je sprovedena posle polulogaritamske transformacije analizom varijanse (ANOVA). t_{max} vrednosti su testirane neparametrijski. Parametrijski 90% intervali poverenja za količnike T/R su se kretali za $AUC_{0\text{-last}}$ od 96,50 do 112,30 % (količnik 104,42 %), za $AUC_{0\text{-inf}}$ od 96,60 do 113,20 % (količnik 104,22 %) i za C_{max} od 97,20 do 108,52 % (količnik 101,36 %). Na osnovu rezultata za AUC, C_{max}, t_{max}, K_{el} i $t_{1/2}$, nema značajnih razlika i dva ispitivana preparata bumetanida su ekvivalentna i po brzini i po količini apsorpcije.

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