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Comparison between Pharmacokinetic and Pharmacodynamic of Single-Doses of Furosemide 40 mg Tablets

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Abstract

This study was to evaluate and compare the pharmacokinetic and pharmacodynamic behavior of two formulations of furosemide (CAS 54-31-9) 40 mg tablets, administered as a single dose to healthy subjects. Plasma concentrations of furosemide were determined with a validated method by liquid chromatography coupled to mass spectrometry (LC-MS/MS). We obtained the parameters: AUC_{0-t}, $AUC_{0-\infty}$, K_{el}, $T_{1/2}$, C_{max} e T_{max}. The following parameters were determined in urine: Sodium, Potassium and Chlorine and the total volume. The 90% confidence intervals for the ratio of C_{max} (93.63-121.92%), AUC_{0-t} (96.80-115.72%) and $AUC_{0-\infty}$ (98.45-117.43%) respectively for test and reference. Statistical analysis of the similarity of the parameters for urinary volume, excretion of sodium, potassium and chlorine and assuming that both formulations reach the same plasma levels, we expect that the pharmacological effect is also the same. Whereas the rate and extent of absorption, both products can be considered therapeutic equivalents.

Keywords: Pharmacokinetic; Bioequivalence; Diuretic action; Furosemide; Dissolution profile

Introduction

Furosemide (CAS 54-31-9) (Figure 1) chemically corresponds to 4-chloro-2-(furan-2-ylmethylamino) - 5-sulfamoylbenzoic acid. This drug has been widely used due to its rapid diuretic effect, especially in acute [1].

Among the loop diuretics, furosemide seems to be more effective for presenting broad dose-response curve, being used in treatment of edema associated with congestive heart failure, liver cirrhosis and chronic kidney disease, including nephrotic syndrome; as an adjuvant in the treatment of acute pulmonary edema; in hypertensive crisis; in mild and moderate hypertension associated with other antihypertensive agents. It can also be used in cases of liver diseases and situations accompanied by hypercalcemia and oliguria caused by kidney failure [1].

It has been reported that furosemide also has a vasodilator action, which seems to be related to decreased sodium retention and increased synthesis of some prostaglandins [1].

The pharmacokinetics of furosemide is well documented in healthy individuals. The absorption is rapid and peak levels occur 60-90 minutes after the dose. It has a high link to plasmatic proteins (97-98%) and it is eliminated by liver and kidney glucuronidation and by renal secretion and filtration. The elimination half-life is relatively fast (0.5-2 h), however, the biphasic elimination kinetics is slow (20-30 h). The interindividual variability of pharmacokinetic behavior of the furosemide is great and it is influenced by underlying disease [2].

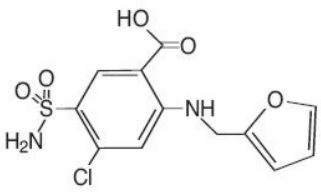

Figure 1: Furosemide chemical structure.

The absorption and elimination of furosemide is relatively fast [3]. The furosemide bioavailability in healthy volunteers is from approximately 50% to 70% for tablet formulations. Its maximum concentration is reached between 1 to 1.5 hours for 40-mg tablets. In patients, the bioavailability is influenced by several factors including underlying disease, and it can be reduced to 30%, for example, in nephrotic syndrome [4].

In a study conducted to evaluate the bioavailability of 40-mg furosemide by using six different formulations in 12 healthy volunteers, the following pharmacokinetic data were found: C_{max} 1404 µg/mL, 1200 µg/mL, 1160 µg/mL, 1938 µg/mL, 1193 µg/mL and 2421 µg/mL; T_{max} 1.79 hours, 1.75 hours, 1.08 hours, 1.71 hours, 1.71 hours and 0.78 hours [5].

Other pharmacokinetic data were cited in study comparing the pharmacokinetic and pharmacodynamic equivalence of two 40-mg-oral formulations of furosemide, where it is observed Tmax of 1.09 and 0.78 hours; C_{max} of 1315.34 and 1473.59 ng/mL and $T_{½}$ of 3.10 and 2.97 hours [6].

This study aimed to evaluate and compare the pharmacokinetic

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and pharmacodynamic behavior of two 40-mg-oral formulations of furosemide, managed in single dose for healthy individuals.

Materials and Methods

The aim of the present work was to assess the bioequivalence/pharmacodynamic of two commercial 40 mg tablets formulations of the drug (Lasix® from Aventis Pharma Ltda, Brazil, manufacturing date in September, 2006, expiry date in August, 2009 and furosemide from Prati, Donaduzzi & Cia Ltda, Brazil, manufacturing date in December, 2006, expiry date in December, 2008).

In vitro study

In order to assess the quality of the pharmaceutical products and to simulate the absorption speed in vivo behavior, it has been carried out the furosemide dissolution profile with 12 tablets of the test and reference formulation by using the Erweka DT-700 device, following the United States Pharmacopeia – USP 31 (2008) method. The used dissolution medium was potassium phosphate buffer pH 5.8, for a volume of 900 mL, with stirring system apparatus 2 (paddle), stirring speed of 50 rpm and temperature of 37ºC. The samples have been collected after the time intervals of 5, 10, 15, 30 and 60 minutes and filtered through quantitative filter paper, obtaining the concentration of 0.044 mg/mL. The analysis was performed on spectrophotometer UV-VIS (Spectro Vision SB-1810S) at wavelength 274 nm as it is recommended by USP 31. The samples were compared toward a standard of a known concentration 0.044 mg/mL diluted in the same dissolution medium.

In vivo study

The study was conducted by taking into account determinations of Hensinki Declaration, as well as Brazilian Resolutions (Resolutions 196/96 of CNS-MS, as well as RDC 103 on May, 8th, 2003 from the National Sanitary Surveillance Agency – ANVISA).

In this study, 28 (twenty-eight) male volunteers aged from 18 to 44 years old have been recruited, who had Body Mass Index (BMI) varying between 19.95 and 26.74, as it is shown in Table 1. In defining the group of volunteers it was decided to use only males, which typically have values for higher blood pressure when compared to females and thus less susceptible to variations in the hypotensive effect caused by the administration of furosemide.

We also found studies showing the great variability in bioavailability and disposition of furosemide. According Grahnén et al., the variation in the absorption of furosemide is a limiting factor in bioavailability studies [12]. This was also a factor that influenced the decision to use only male subjects in this study because, according to a study conducted by Carvalho, the use of women in bioequivalence studies could trigger changes in the analytical results of the study, this probably occurs due to biochemical differences between males and females and also the use of oral contraceptives by women [13].

Variables	N	Average	Median	SD	Minimum	Maximum
Age (years)	28	26.71	23	8.51	18	44
Weight(Kg)	28	70	69	6.88	59.10	87.50
Height (m)	28	1.73	1.73	0.05	1.61	1.84
BMI	28	23.38	23.09	2.13	19.95	26.74

SD = Standard Deviation

Table 1: Descriptive statistical table of the studied population.

The Study protocol and free and clarified consent term has been submitted and approved by the Ethical Committee for the use of human beings in scientific research at Assis Gurguz College (Cascavel, Brazil), which is recognized by the CONEP (National Health Council/MS) and all the volunteers who have participated in the research applied for it spontaneously in order to take part in the Bioequivalence Study, even though it offered them no therapeutic benefits. The formal authorization for this participation has been granted and clarified before the beginning of the study by the volunteer, after all his/her doubts had been explained. The volunteers' inclusion in the study was determined by their health state, confirmed through medical history, physical exercises and laboratory exams. This screening aims to increase the safety for the volunteer and to avoid the presence of pathological processes which might interfere in the drugs pharmacokinetics and that could provide incorrect results.

The selected volunteers did not present heart, liver, kidney, pulmonary, neurological, gastrointestinal and hematological diseases, which were evaluated in the medical exam, electrocardiogram and laboratory exams: fasting glycemia, urea, creatinine, oxalacetic transaminase, pyruvic transaminase, alkaline phosphatase, total bilirubin, albumin, total protein, triglycerides, total cholesterol, hemoglobin, hematocrit, total and differential white blood cell count and urine test. The volunteers had negative serological tests for HIV, hepatitis B (Anti-HBs, HBsAg, HBc IgG, HBc IgM) and hepatitis C (Anti-HBC) and β-HCG.

The conducted study was open, randomized and crossover, with two hospitalizations of at least 7 (seven) interval days (crossover 2×2). The interval between the hospitalizations followed the interval of 7 half-lives required by ANVISA (National Sanitary Surveillance Agency) in order to ensure that there was no residual effect of the first drug administrated, which would artificially increase the second period results. The formulations were administrated after 11 hours of fasting, where the volunteers received a single 40 mg dose of each formulation of Furosemide, which is produced and donated by Prati, donaduzzi & Cia Ltda or Lasix® reference formulation with 200 mL of water as randomization. In order to maintain the standardization of the treatment groups, food was not allowed during the three hours that followed the drug administration, when breakfast, then, was provided; a standard lunch, an afternoon snack and dinner were consumed at 6.0; 10.0 and 13.0 hours. after the administration, respectively. No other food was allowed during the hospitalization period. It was allowed liquid ingestion an hour after the administration, however, xanthine-containing drinks such as, tea, coffee and coke were avoided.

For the determination of drug plasma levels, 8 mL blood samples have been collected through heparinized catheter inserted into the superficial vein of the forearm, considering that the blood collections occurred 0 (pre-dose), 0.17; 0.33; 0.5; 0.67; 0.83; 1.0; 1.17; 1.33; 1.5; 1.75; 2.0; 2.5; 3.0; 3.5; 4.0; 6.0; 8.0; 10.0; 12.0 and 15.0 hours after the administration of each formulation in different times. For the Pharmacodynamic analysis of the drug, all the eliminated urine was collected in the following periods after the administration: 0 to 4.0; 4.0 to 8.0 and 8.0 to 12.0 hours.

There was the verification of systolic and diastolic blood pressures and heart rate in times 0.5; 1.0; 1.5; 2.0; 4.0; 6.0; 8.0; 10.0; 12.0 and 15.0 hours after the administration.

Sample analysis

The blood samples were centrifuged at 3000 rpm per minutes at 4ºC and the plasma was transferred to cryogenic tubes, stored in a freezer at -20ºC until the use in the drug quantification test.

The urine samples were collected in suitable individual containers. After the collection interval had finished, such containers with the urine were carefully measured by using a suitable beaker, recording the total volume for each time interval and aliquoted (20 mL) for the following electrolytes analysis (sodium, potassium and chloride).

Plasma concentrations of furosemide were determined through the method validated by liquid chromatography-mass spectrometry (LC-MS/MS) using chlorthalidone as internal standard. The mass spectrometer was equipped with negative electrospray source (ESI-) by usingmultiple reaction monitoring (MRM) mode, monitoring the transitions 328.4>284.9 and 336.4>190.0 for furosemide and chlortalidone, respectively.

The plasma samples containing furosemide were thawed at environmental temperature and stirred manually for 5 seconds. A total of 200µL of plasma has been transferred to eppendorf tube and added 50µL of internal standard solution (4000 ng/mL) and 50µL of HCl 4M, which were shaken for a minute in a microtube shaker. Then, 1.5mL of diethyl ether was added and the tubes were shaken again in microtube shaker for 10 minutes. Afterwards, the samples were centrifuged for 10 minutes in 10,000 rpm under the temperature of 4ºC. After freezing the samples, the supernatant was transferred and evaporated under nitrogen until the drying at 45ºC. The residue was reconstituted with 200µL of mobile phase. The analysis was performed in a flow of 0.7 mL/min with injection volume of 20 µL and the samples were kept at 4ºC. The mobile phase consisted of Solution A (consisting of acetonitrile and acetic acid 0.1% in the proportions 90:10 v/v): Methanol (90:10 v/v). The chromatographic separation was carried out in Synergy fusion C_{18} column (50×4.6 mm, 4µm) Phenomenex® fitted with pre-column with the same filling material under the temperature of 40ºC.

Retention times of analysis were 1.29 minutes for Furosemide and 1.26 minutes for the Chlortalidone internal standard, being the total running time of 2 minutes. The method was validated by the determination of the following parameters: specificity, linearity, recovery, accuracy, precision, lower limit of quantitation (LLOQ) and stability studies [7].

Pharmacokinetics, pharmacodynamic and statistical analysis

It has been obtained the parameters: AUC_{0-t}, $AUC_{0-\infty}$, K_{el}, $T_{1/2}$, C_{max} e T_{max}. The observed maximum plasma concentration (C_{max}) and the time required in order to reach this concentration (T_{max}) were obtained directly from the concentration curves of the volunteers. The area under the concentration curve of furosemide in plasma versus time from 0 to 15 hours (AUC_{0-t}) was calculated by applying the trapezoidal rule [8]. Extrapolating this, $AUC_{0-\infty}$, was calculated by adding the value C_t/K_{el} to AUC_{0-t}. Statistical analysis was conducted after logarithmic transformation based on additive model for all values of AUC and C_{max} [8].

The rate constant of first-order terminal elimination (K_{el}) was estimated by the linear regression slope, calculated by the method of least mean square, the natural logarithm of concentration versus time. The half-life ($T_{1/2}$) was originated from this rate constant ($\ln(2)/K_{el}$). It has been used the variance analysis (ANOVA) for crossover model. The formulations are considered equivalent bioavailability, when the confidence interval of 90% of reason between the geometric means (T/R) of AUC and C_{max}, related to furosemide, were within the range of 80-125% (bioequivalence interval), in accordance with the laws of ANVISA [9].

Through the selective/automated Electrode method, the following parameters were determined in Urine: Sodium, Potassium and Chlorine. In addition to these, it has been measured the total volume excreted in each period. For these data, it has been tested the equality of means of the drug tests and reference toward the times 0 to 4.0 h, 4.0 to 8.0 h and 8.0 to 12.0 h for the four parameters, using test t and the significance level was 5%.

Pharmacokinetics/pharmadynamic and statistical analysis were carried out by using the softwares GraphPad Prism® version 4.0, WinNolin® version 5.0.1, EquivTest® version 2 and Microsoft Excel®.

Results

Dissolution test of tablets of furosemide, described on the USP 31, recommends that not less than 85% of the drug must be dissolved in 60 minutes. The dissolution profile (% dissolved of furosemide versus time) is shown in Figure 2.

The evaluation of the dissolution profile obtained from the products in study indicates that the test and reference drug are similar, showing that the formulations are very homogeneous. The dissolution was approved, for not less than 85% of the dissolution occurred in 60 minutes. In order to complement the comparative study of the dissolution profile of the drugs consisted of furosemide; the independent model method was applied, where the difference factors were calculated ($f1$) finding the value of 2.88 and the similarity ($f2$) finding the value of 74.76.

The analysis through this method showed that the test product is similar to the reference drug, since the factor of difference was lower than 15 and the similarity was higher than 50.

The study population was to be 28 volunteers, however, it consisted of 27 (twenty-seven) healthy male volunteers at the age from 18 to 44 years old (mean ± standard deviation: 26.71 ± 8.51 years old) with a height from161 cm to 184 cm (173± 5 cm), weighing between 59.1 kg to 87.5 kg (70 ± 6.88 kg).

Furosemide formulations have been well tolerated in administrated dose. The biochemical parameters remained unchanged and within the reference standard until the end of the study. Some adverse events

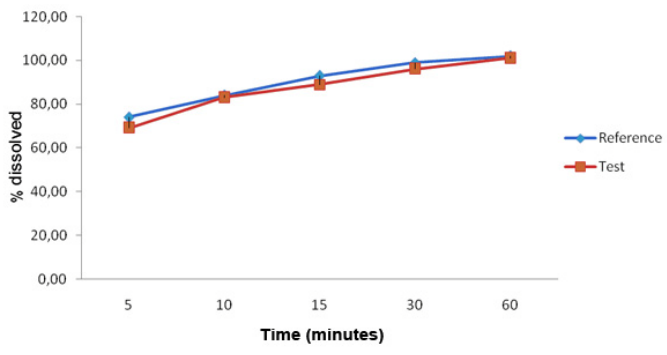

Figure 2: Profile of furosemide dissolution.

were detected: one volunteer reported headache in the two periods of confinement. The symptoms were mild and probably not related to furosemide.

The calibration curve contemplated plasma concentration range from 10 to 2000 ng/mL, considering that the straight-line equation was obtained through linear regression, which has shown linear correlation coefficient equal to 0.9915. The limit of determined quantification corresponded to 10 ng/mL and the detection corresponded to values ≤ 2ng/mL. The mean recovery of furosemide and chlorthalidone were 95.8% and 96.9% respectively. The results of matrix effect evaluation were within the acceptable limits as show in Figure 3. A typical chromatogram obtained by the proposed LC method, with the resolution of the symmetrical peak corresponding to furosemide and chlorthalidone, is shown in Figure 4. The quality controls used for method validation and for the study implementation were determined in 30 ng/mL (Low Quality Control), 750 ng/mL (Average Quality Control) and 1500 ng/mL (High Quality Control). The intra-day accuracy of the method was between 93.5 and 104.9% with a precision of 2.2 – 12.3%. The inter-day accuracy was between 96.5 and 101.9% with a RSD of 1.9 – 6.4%. The data portray that the method possesses adequate repeatability and reproducibility. The plasma samples were stable for at least 52 days at -20 °C (long-term) and also after three freeze-thaw cycles, demonstrating that human plasma samples could be thawed and refrozen without compromising the integrity of the samples. furosemide was stable in neat plasma for up to 4h at room temperature (short-term). The results demonstrated that extracted

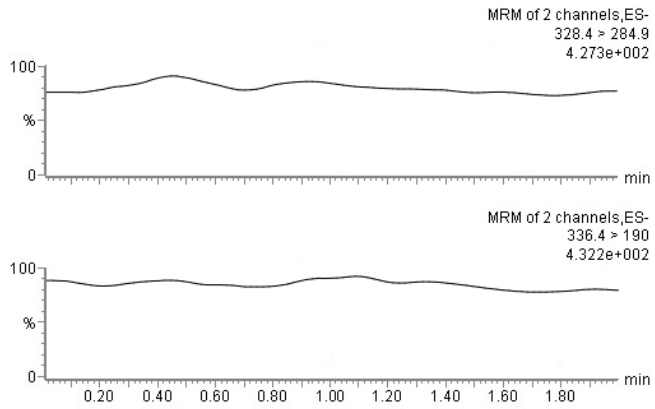

Figure 3: Blank plasma chromatograms for furosemide and chlorthalidone.

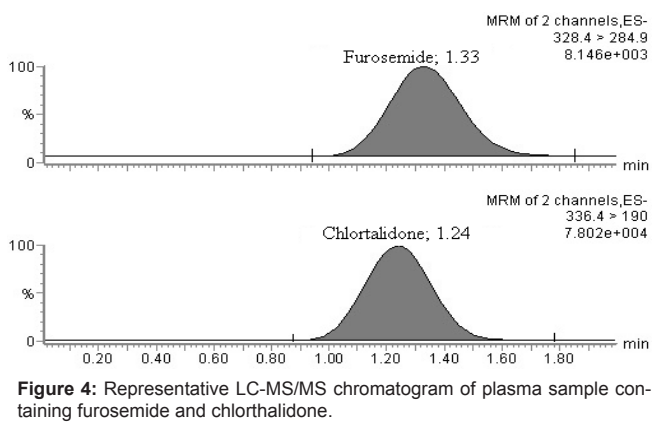

Figure 4: Representative LC-MS/MS chromatogram of plasma sample containing furosemide and chlorthalidone.

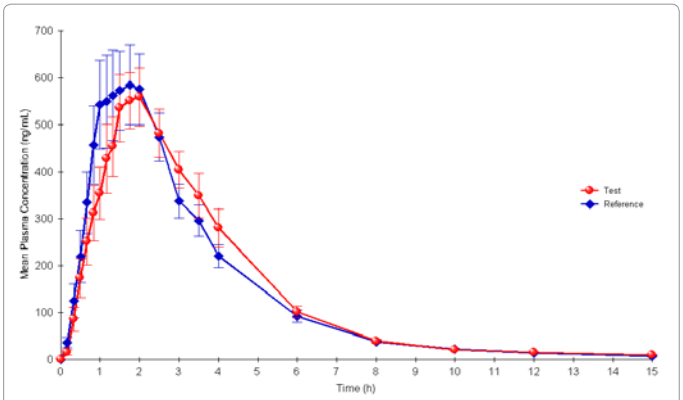

Figure 5: Curve of mean plasma concentrations ± standard error of test and reference drugs.

samples could be analysed after keeping in the autosampler for at least 51h with an acceptable precision and accuracy.

The curve of mean plasma concentrations versus time of test and reference drugs are in Figure 5. It is verified that the absorption, distribution and elimination of drugs are similar.

In Table 3 shows pharmacokinetic parameters along with confidence intervals of 90% of 40mg furosemide.

Discussion

The specificity, linearity, recovery, accuracy, precision, lower limit of quantitation (LLOQ) and stability studies of the assay were sufficient for concentration determination the plasma bioequivalence of furosemide in healthy volunteers.

Comparing the presented values, we can see the similarity of the data found in respect to the parameter of $AUC_{0-\infty,}$ both presenting with values ranging from 2038.5 to 2233.8ng/mL. For T_{max} and C_{max} the found values for different authors showed some variations generating ranges of 0.84 to 2.2 and 763.5 to 1584.72, respectively [10].

Articles present in their study C_{max} values of 1584.72 ± 654.14 and 1438.3± 632.47 ng/mL for the test and reference formulations, respectively, that is, results far higher than those of this study (879.32 ±345.03 and 884.36 ±,501.15) [6,10,11].

In regard to T_{max} pharmacokinetic parameter, it has been observed that there was no disparity between the obtained in this study compared to the others found in literature [6,10,11].

For $T_{1/2}$ parameter, it has been observed a larger range, i.e., a higher difference between the results found between the different authors. This might be related to different chronograms of collections used in each research. It was used the following chronogram of collection 0.25; 0.5; 0.75; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5; 5.0; 6.0; 7.0; 8.0; 10.0 and 12.0 hours [10]. As well as in another article the blood samples were collected at the times 0.33; 0.66; 1.0; 1.33; 1.66; 2.0; 3.0; 4.0; 5.0; 6.0; 7.0; 8.0; 10.0 and 12.0 hours [6]. This research observed more collection points than the mentioned authors, as well as it reached a longer period of elimination of the drug, 0.16; 0.33; 0.5; 0.66; 0.83; 1.0; 1.16; 1.33; 1.5; 1.75; 2.0; 2.5; 3.0; 3.5; 4.0; 6.0; 8.0; 10.0; 12.0 and 15.0 hours. As $AUC_{0-\infty}$ consists of representative estimated value of the drug absorption total extent and that AUC_{0-t} represents the extent of the quantified absorption, the relation between these two parameters was higher

	Source of Variation	*df*	SS	MS	Computed F	P-value
AUC0–t						
	Subject	1	0.0431	0.0431	0.1508	0.7010
	Subject (sequence)	25	7.1513	0.2861	7.7731	0.0000
	Period	1	0.0433	0.0433	1.1776	0.2882
	Formulation	1	0.0455	0.0455	1.2373	0.2766
	Error	25	0.9200	0.0368	-	-
	Total	53	8.2032	-	-	-
$AUC_{0-\infty}$						
	Subject	1	0.0222	0.0222	0.0913	0.7651
	Subject (sequence)	25	6.0787	0.2431	6.7752	0.0000
	Period	1	0.0709	0.0709	1.9759	0.1721
	Formulation	1	0.0558	0.0558	1.5552	0.2239
	Error	25	0.8972	0.0359	-	-
	Total	53	7.1248	-	-	-
C_{max}						
	Subject	1	0.0697	0.0697	0.1856	0.6703
	Subject (sequence)	25	9.3831	0.3753	4.6666	0.0001
	Period	1	0.0591	0.0591	0.7345	0.3996
	Formulation	1	0.1014	0.1014	1.2609	0.2721
	Error	25	2.0107	0.0804	-	-
	Total	53	11.6240	-	-	-

Table 2: Analysis of variance table of logarithmically transformed AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} of test and reference of 40mg furosemide tablets after single-dose administration in healthy male volunteers (N = 27).

Pharmacokinetic Parameters	Test (T)		Reference (R)		Mean Ratio** T/R	I C 90% for Mean Ratio**
	Mean*	SD	Mean*	SD		
T_{max} (h)	1.82	0.81	1.54	0.75	-	-
C_{max} (ng/mL)	879.30	345.00	884.30	501.10	106.84%	93.63% - 121.92%
K_{el}	0.24	0.14	0.24	0.12	-	-
$T_{1/2}$ (h)	4.70	5.36	3.86	2.70	-	-
AUC_{0-t} (ng.h/mL)	2137.70	795.40	2107.30	1058.50	105.83%	96.80% - 115.72%
$AUC_{0-\infty}$ (ng.h/mL)	2233.80	768.70	2183.00	1061.00	107.52%	98.45% - 117.43%

* Arithmetic mean; ** Geometric mean; IC = Confidence intervals; SD = Standard deviation

Table 3: Pharmacokinetic parameters obtained after study using single dose of 40mg furosemide tablets.

than 80%, showing that in this assay the efficiency of the experimental design, comparing with the study, the AUC_{0-t} values found in this study were lower (2137.78 for the test drug and 2107.38 for the reference test) [11], as well as in $AUC_{0-\infty}$ parameter, which also were lower than other articles and comparable with one of another study [10].

The statistical analysis, accomplished through ANOVA, revealed the absence of any formulation effect with regard to AUC_{0-t}, $AUC_{0-\infty}$, and C_{max}. No significant sequence effect was found for all of the pharmacokinetic parameters. An ANOVA of the pharmacokinetic parameters is shown in Table 2. The statistical comparison of AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} found no significant difference between the test and reference formulations of 40mg furosemide tablets.

The point estimates for the mean ratio of test/reference product of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were 105.83%, 107.52% and 106.84%, respectively, and the parametric 90% CIs for AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were 96,80% to 115,72%), 98,45% to 117,43%), and 93,63% to121,92%, respectively (Table 3). Statistical analysis of bioequivalence of both formulations described in Table 3, it has been verified that the test drug is bioequivalent to the reference drug, because the confidence interval of the parameters are within the bioequivalence ranges defined by the regulatory agency.

Parameter	Time								
	0 - 4 h			4 - 8 h			8 - 12 h		
	Mean		p-value	Mean		p-value	Mean		p-value
	T	R		T	R		T	R	
Volume (mL)	1868.93	1701.79	0.2371	307.14	323.21	0.7533	219.82	163.57	0.2555
Sodium (mEq)	127.63	115.13	0.0714	43.29	44.91	0.8267	35.06	27.42	0.3062
Potassium (mEq)	18.94	16.59	0.9498	8.82	10.13	0.3899	10.56	9.35	0.5598
Chlorine (mEq)	244.99	232.87	0.2872	23.82	22.48	0.8200	11.12	6.65	0.2264

Table 4: Statistical Analysis of urine parameters volume, sodium, potassium and chlorine for test (T) and reference (R) formulation.

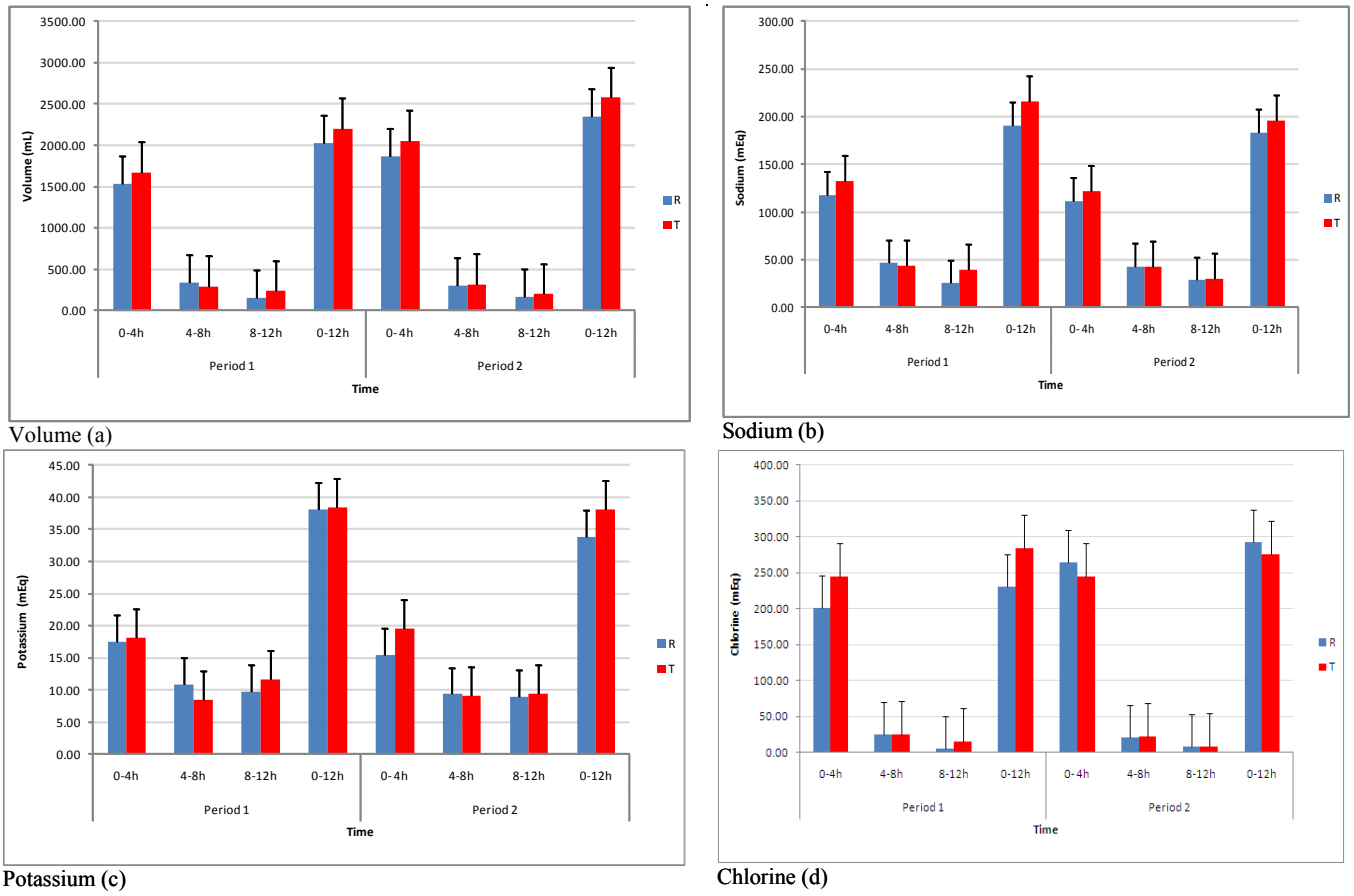

Figure 6: Urine volume (a) and sodium excretion (b), potassium (c) and chlorine (c) after the administration of furosemide test (T) and reference (R) formulations.

Furthermore, the power for this study was >99% based on the mean values of AUC_{0-t}, $AUC_{0-\infty}$, for C_{max} the power was 87%.

In Table 4 is presented the data obtained from the Volume, Sodium, Potassium and Chlorine parameters, obtained by collecting urine.

It has been observed that independently of the analyzed salt the average quantity referred to the test drug is always higher when compared to the reference drug, however, it has been verified that the non existence of significant differences at the level of 5% of significance between the means of the test and reference drug, i.e., the mean values of each time interval are similar for all the analyzed parameters (total volume, sodium, potassium and chlorine). In Figure 6 is presented the pharmacodynamic parameters of the furosemide excreted urine, divided into two periods of hospitalization.

The total excreted volume of urine in the first period was lower compared with the second period; it has been also observed that the greater part of the excreted volume was at the time of 0-4 hours, about 75% of the total volume. Similar result occurred for sodium salt. For chlorine parameter, about 88% of the excreted total occurred at the first 4 hours and it has been found a very low index in the last collection interval (8-12 hours). It was verified that for potassium parameter it also occurred higher elimination in the first collection interval, about 48%, and remained virtually constant in the other collection intervals.

The action of loop diuretics, as furosemide is classified, occurs through the inhibition of the pump that accounts for the reabsorption of sodium chloride and potassium chloride in the thick ascending loop of Henle glomerular. The main action of antidiuretic hormone and renal medullary interstitial in order to concentrate urine occurs in this place; where about 20 to 30% of the filtered sodium is reabsorbed. The reabsorption blockade, mainly of sodium, in this place, causes a very pronounced diuretic effect, causing a higher volume of diuresis coincidently with the T_{max} period. As the losses of cations Na^+ and K^+ are accompanied by chloride, it is natural that the volume of the excreted chlorine excreted coincided with the period of higher diuresis volume, i.e., during the T_{max} period. The renal balance, in an attempt to reverse sodium loss, gradually starts an activity of Na-K pump, changing the sodium reabsorption by the elimination of potassium, thus, as it has been seen in dosages, during the periods following the T_{max}, the amount of potassium removed is expanding, for this purpose is that furosemide is considered, when used chronically, as potassium-wasting.

The bioavailability comparison of different formulations of a same active ingredient in the same concentration guarantees its interchangeability. Assuming that both formulations reach the same plasma level, we expect that the pharmacological effect is also the same. Considering the rate and the extent of absorption as it is required by the regulations of Food and Drug Administration and ANVISA (National Agency for Sanitary Surveillance), both products can be considered bioequivalent and, therefore, therapeutic equivalents in medical practices.

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