Technological development of 40mg furosemide tablets: equivalence and bioavaibility study in dogs[1]

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ABSTRACT.- Lima E.Q., Albuquerque M.M., Rodrigues O.G., Alencar J.R.B., Medeiros F.P.M. & Neto P.R. 2007. **Technological development of (40mg) furosemide tablets: a equivalence and bioavaibility study in dogs.** *Pesquisa Veterinária Brasileira 27(11):462.466.* Unidade Acadêmica de Medicina Veterinária (UAMV), Centro de Saúde e Tecnologia Rural (CSTR), Universidade Federal de Campina Grande (UFCG), Av. Acadêmica s/n, Santa Cecília, Cx. Postal 64, Patos, PB 58708110, Brazil. E-mail: onaldo@cstr.ufcg.edu.br

Furosemide (40mg) was administered to 20 street dogs, 10 males and 10 females, in two different pharmaceutical forms: (1) compressed furosemide 40mg formulated at the Federal University of Pernambuco (UFPE-tablet), and (2) a commercial formulation with equal bioequivalence produced by the Laboratory for Pharmaceutical Technology of Pernambuco State (LAFEPE), the LAFEPE-furosemide. The study aimed to evaluate the kinetics of dissolution of the UFPE-tablet in order to analyze the behavior of bioavailability of the best formulation for veterinary use. The plasmatic concentrations of furosemide for the determination of parameters of pharmacological kinetics were analyzed by high-performance liquid chromatographic method (HPLC). The *in vitro* study accomplished through physiochemical analyses demonstrated that the formulas of the furosemide tablets attained the pharmaceutical requirements in agreement with USP 23 and the Brazilian Pharmacopoeia. The evaluation accomplished in dogs with UFPE-tablets given in only dose demonstrated uniformity in blood levels indicating stability in maintenance of the pharmaceutical formulation and efficiency in absorption of the active compound. These values are not significantly different in relation to the 5% confidence limit. Regarding maximum concentration (T_{max}) time and global bioavaibility assessed by AUC means, there were no considerable differences as well. UFPE-furosemide displayed 743.492μg/mL.h as AUC average value whereas LAFEPE-furosemide had an average of 537.284μg/mL.h.

INDEX TERMS: Furosemide, bioavaibility, lyoavailability, dogs.

RESUMO.- [**Desenvolvimento tecnológico da furosemida comprimido (40mg): estudo da equivalência e biodisponibilidade em cães.**] Furosemida (40mg) foi administrada a 20 cães de rua (cães SRD), 10 machos e 10 fêmeas, em duas formas farmacêuicas distintas: (1) furosemida comprimido 40mg formulada na Universidade Federal de Pernambuco (UFPE-comprimido) e (2) uma formulação comercial bioequivalente produzida pelo Laboratório de Tecnologia Farmacêutica do Estado de Pernambuco (LAFEPE-furosemida). O estudo objetivou avaliar a cinética de dissolução do UFPE-comprimido para analisar o comportamento da liodisponibilidade dos comprimidos buscando a melhor formulação para uso veterinário. As concentrações plasmáticas de furosemida para determinação de parâmetros farmacocinéticos, foram analisadas por cromatografia líquida de alto desempenho (HPLC). O estudo *in vitro* realizado através de análises físicoquímicas demonstrou que as fórmulas dos comprimidos de furosemida preencheram os requisitos farmacêuticos de acordo com USP 23 e a Farmacopéia Brasileira. Avaliações realizadas em cães da formulação UFPE-comprimidos administrados em dose única, demonstrou uma uniformidade nos

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níveis sangüíneos indicando estabilidade na manutenção da forma farmacêutica e eficiência na absorção do princípio ativo. Estes valores não são significativamente diferentes em relação ao limite de confiança de 5%. Em relação a concentração de máximo (Tmax) e ao tempo de biodisponibilidade global avaliados por meios de AUC, não houve nenhuma diferença considerável. O comprimido UFPE-furosemide exibiu AUC de 743.492µg/mL.h e o LAFEPE-furosemide teve uma média de 537.284µg/mL.h.

TERMOS DE INDEXAÇÃO: Furosemida, biodisponibilidade, liodisponibilidade, cães.

INTRODUCTION

Furosemide is an anthranylic acid derivative commonly used in man as a potent tubule diuretic (Pontc & Schoenwald 1990, Awad et al. 1992). It is frequently used in human edema treatment associated with congestive heart failure, hypertension and renal insufficiency. In domestic animals furosemide is used to treat various forms of edema in "azoturia", to reduce space-filling lesions and as a prophylactic measure of epistaxis in racing horses (Roberts et al. 1978, Tobin 1978). Furosemide has a protective effect against bronchoconstriction caused by several factors including exercise (Maxson et al. 1995), and has been used illegally to 'dilute out' prohibited drugs (Stevenson et al. 1990, 1994, Sweeney et al. 1990). Therefore, its use in race horses should be monitored (Stevenson et al. 1994).

Nowadays one of the basic tasks of drug formulation is to develop an already existing dosage form in a way that makes the best drug release possible under the given circumstances, in other words, enhancing bioavalailability (Dhanaraju 1998, Sznitowska 2001, Matsuura et al. 2002). Furosemide pharmacokinetics has been studied previously in several animal species, particularly horses (Roberts et al. 1978, Chay et al. 1983, Singh et al. 1990, Dyke et al. 1996), but also in dogs (Verbeeck et al. 1981, Hirai et al. 1992, Rahman et al. 2001) and rats (Hammarlund & Paalzow 1982).

Furosemide pharmacokinetics demonstrates 11-90% of oral absorption, 0.3-3.4% of half life, 60% of elimination in the unaltered form and 40% in the metabolized form (Hammarlund 1984, Goodman 1996). According to Johnston (1984), furosemide is rapidly absorbed in the conventional tablet form with high diuretic peaks in dogs after one hour of administration (Chungi 1979, Jitka Huclov 2005). In this kind of study, it is assumed that the success or failure of the pharmaceutical treatment depends largely on certain factors, which are not limited to the usual pharmacodynamics or pharmacokinetics knowledge present in pharmacology compendia used in human medicine.

This study aims to report the technological development of 40mg furosemide tablets produced at Pernambuco's Federal University (UFPE-tablets) in comparison with the ones developed *in vitro* and *in vivo* at Pernambuco State Pharmaceutical Laboratory (LAFEPE- furosemide), using street dogs administration model to meet therapeutic needs in Veterinary Medicine practice.

MATERIALS AND METHODS

Experiment place and technological process

This study was carried out in the Laboratory for Pharmaceutical Technology of Pernambuco (LAFEPE) at Federal University of Campina Grande (UFCG) laboratories. The technological development of UFPE-tablets was based on Good Practices of Manufacture (BPF in portuguese). In the pre-formulation study the following materials were used: diluents (starch, calcium phosphate, lactose), agglutinants (arabic gum, PVP K30, carboxymethylcellulose), lubricants (magnesium stearate, powder), furosemide (active principle) and moisture (hydroholic solution). The compression process by humid via was used in tablets production.

Animals, drug administration and blood sampling

The animal selection was done with a sample of twenty dogs (street-dog), with standard weight and age. Sixteen healthy dogs with ages varying from 3-5 years and weighing between 20 and 30 kilos were selected, 10 males and 6 females. The dogs underwent clinical evaluation during a 30-day-period at the Federal University of Campina Grande (UFCG) Kennel. They were submitted to a twelve-hour fasting after the last meal of the previous day with no water restriction. A single 80mg dose (two 40mg furosemide-tablets 5 per cent w/v, Hoechst Animal Health, Bucks, UK) of the formulations under study (UFPE-tablets and LAFEPE furosemide) was administrated in the morning. The dogs were monitored during the whole study and had the same accompaniment.

Furosemide analysis

6mL of blood samples were collected in heparinized tubes in 0, 20min, 40min, 60min, 90min, 120min, and 180min intervals. The plasma, which was protected from the light until furosemide analysis, was separated by a centrifugation of 3,500 rpm for 10 minutes kept at a -20ºC temperature. All the samples of the same animal were analyzed in the same day to avoid variation among analysis. Furosemide plasma concentration was determined by High Pressure Liquid Chromatography (HPLC).

Analytic methodology

The quality of the products under study was evaluated in two stages, according to the 4th edition of the Brazilian Pharmacopeia (Brasil, Ministério da Saúde 1988) and the 23rd edition of the United States Pharmacopeia (USP 1995). At first, physicochemical assay of average weight, hardness, friability and disintegration were fulfilled. Subsequently, essays on assay, content uniformity and liberation kinetics were carried out. These stages determine the *in vitro* study.

Plasma samples preparation

Furosemide concentration in plasma samples was determined by HPLC, HPLC acetonitrile degree and Merck methanol were used as solvents. The mobile phase was a mixture of water: acetonitrile (70:30) and the pH was adjusted with NaOH 0.2N or glacial acetic acid (1:100) to 6.5. The HPLC system chromatographic conditions consist of a solvent system; a 20 µL Rheodyne valve; an UV detector; an integrator; ODS 25µm spherisorb waters, a 1mL/min air flow and a 235 nm wave length.

Extraction of samples procedure

The following procedure was used in order to determine the furosemide in plasma samples. Firstly, 0.2mL of plasma was taken and 2.4µg/mL of fenacetine in methanol was added. This material was stirred in vortex for 40 seconds and then centrifuged at 3000 rpm for 12 minutes. Secondly, the methanol layer was decanted and

centrifuged for 5 minutes. A 1mL portion was transferred to a volumetric balloon concluding the mobile phase. A 20μL volume was injected. A threefold procedure was carried out. The standard curve (n=6, r=0.9899) was used to calculate the unknown furosemide plasma concentrations. The validation of the analytic methodology employed furosemide methanoic solutions and plasma furosemide. The feasibility of analysis reproduction was assessed through the extraction of six plasma samples with concentrations of 200, 240, 280, 320, 360 and 400μg/mL. The following parameters were compared by means of variance analysis (ANOVA): maximum plasma concentration (C_{max}), time for reaching maximum concentration (T_{max}) and area under the time of maximum concentration curve (AUC).

RESULTS AND DISCUSSION

The *in vitro* study carried out through physicochemical essays showed that the furosemide tablet formulations met the pharmaceutical requirements according to USPC (1995) and Brazilian Pharmacopoeia, 4th edition (Table 1). The data concerning the studied dissolution formulation of Furosemida tablets reached the monography requirements - more than 65% was dissolved after 30min (Table 2). Still, the profiles showed that the products reach 65% of dissolution *in vitro* in 10 minutes after the beginning of the test and show a similar profile in the active principle liberation (Fig.1).

The furosemide in the dog's plasma was carried out as a flowing stage (Table 3). The calibration line was determined with the results obtained from the standard plasma furosemide solutions and underwent statistic treatment, with (R = 0.9942) and p <0.0001. The correlation coefficient was R = 0.9899 (Fig.2).
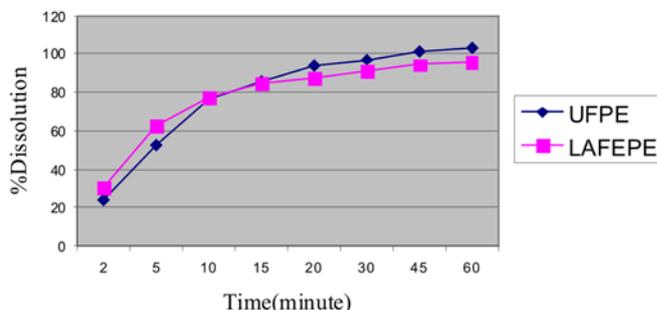

Fig.1. UFPE-tablets and LAFEPE-furosemide dissolution profile *in vitro* according to USP (1995) methodology.

Table 3. Street dogs' blood plasma releasing area values for UFPE-tablets and LAFEPE- furosemide

Animals	Time in minutes					
	20	40	60	90	120	180
UFPE 1	*	*	*	*	*	*
UFPE 2	150.5351	170.9191	224.2029	347.1068	321.1184	207.6041
UFPE 3	163.5749	254.8666	290.8333	244.5177	318.1086	201.8267
UFPE 4	94.33527	251.5243	265.5201	265.0487	272.3010	209.6831
UFPE 5	163.4254	193.0297	255.0887	272.2891	322.0473	267.6248
UFPE 6	159.8313	213.7495	392.6931	451.9548	387.9163	255.5401
UFPE 7	Branco	Branco	Branco	Branco	Branco	Branco
LAFEPE 1	120.4226	167.6343	187.1673	142.0732	174.3153	156.7631
LAFEPE 2	*	*	*231.1035	*	*288.6086	*
LAFEPE 3	128.3341	265.6678	208.1480	271.5379	265.5710	223.6668
LAFEPE 4	136.3287	157.1767	258.8154	205.9522	203.5334	196.2784
LAFEPE 5	145.8162	240.4815	246.3531	245.449	189.0860	149.6032
LAFEPE 6	175.7005	224.2765	207.2939	252.9419	248.9107	276.9624
LAFEPE 7	Branco	Branco	Branco	Branco	Branco	Branco

(p <0,05)

* These values were excluded due to results related to some technical failures such as hemolysis and losses in sample extraction process.

Table 1. Pharmaceutical equivalence results and physicochemical control by UFPE-tablets and LAFEPE-furosemide

Parameters analyzed	Specification	UFPE- tablets	LAFEPE-furosemide
Weight (mg)	200 mg ± 5%	206.6	203.2
Hardness (Kgf/cm^2)	> 3,5 (kgf/cm^2)	8.15	7.11
Friability (%)	< 1,5 %	0.18	0.15
Disintegration (min)	< 30 minute	8 min	5 min
Humidity (KF)	< 2,0%	0.83	1.23
Assay	(90-110%)	100 %	98.75%
Dissolution (%)	> 80%	103.01	95.41
Uniformity Content	85-115%	98.63	96.68

Table 2. UFPE tablets and LAFEPE-furosemide dissolution data *in vitro* according to USP 23 methodology

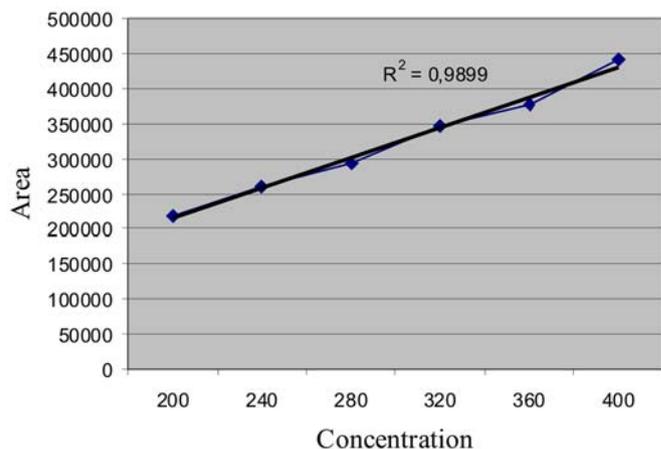| Time (minutes)	% Dissolution	
	UFPE-tablets	LAFEPE-furosemide
2	24.10	30.20
5	52.50	62.83
10	76.80	77.62
15	86.00	84.57
20	94.00	87.64
30	97.10	91.21
45	101.58	94.44
60	103.01	95.41

(p <0.05)

Fig.2. Standard furosemide calibration line in High Precision Liquid Cromatography (HPLC).

The bioavailability evaluation according to study specification entails the determination of pharmacy absorption quantity in 20, 40, 60, 90, 120 and 180 min intervals, i.e., the velocity in which this process happens (United States Pharmacopoeia 1990, Ritchel 1992) (Table 4). Since bio-equivalence is the bioavailability comparative study between two phar-

Table 4. Pharmaceutical equivalence comparison between UFPE-tablets and LAFEPE-furosemide (40mg). Data on *in vivo* kinetics sample results extracted from the dogs' (SRD) blood plasma

Dogs	$t_{a½}$	$t_{e½}$	AUC_{0-t}	$AUC_{t-\mu}$	$C_{máx.}$ cal.	$T_{máx.}$
UFPE 2	0.2818	1.5891	661.57	475.96	350.784	1.35
UFPE 3	0.5175	1.5235	751.12	443.59	296.820	1.24
UFPE 4	0.2439	4.0482	659.71	1224.60	273.299	1.22
UFPE 5	0.4552	3.7445	703.26	1445.74	295.051	1.77
UFPE-6	0.3778	1.7982	941.80	662.95	401.957	1.23
Mean	0.3012	2.5407	743.492	850.568	288.390	1.362
DS	0.0690	1.2463	116.976	456.972	13.099	0.234
CV	22.92%	49.054%	15.733%	53.725%	4.542%	17.183%
LAFEPE 1	0.1794	6.5311	465.65	1477.09	186.757	1.09
LAFEPE-3	0.1548	5.1209	675.84	1652.42	286.055	1.02
LAFEPE 4	0.1723	2.5545	559.57	551.33	248.622	0.88
LAFEPE 5	0.2478	2.1829	564.86	471.15	247.778	0.97
LAFEPE 6	0.2632	21.5724	420.50	7746.69	249.066	1.60
Mean	0.2301	4.097	537.284	1037.998	261.248	1.112
DS	0.0446	2.083	99.026	613.318	21.485	0.283
CV	19.38%	50.84%	18.43%	59.087%	8.224%	25.478%

(p <0.05)

maceutical forms or products containing the same pharmacy in the equal doses, adequate parameters, which describe the absorption process, must be chosen (Jackson 1994). In the bioequivalence study, the pharmacokinetic parameters calculus is usually obtained from blood line pharmacy concentration versus time after administration of the formulations under study. In this case, the area under the curve (AUC), the maximum blood concentration (C_{max}) and the time in which the C_{max} is reached are determined. As observed in Table 4, furosemide displays short plasmatic half time, being liberated in about 60-80% in the blood plasma.

The plasmatic concentration released in the street dogs plasma in both UFPE-tablets and LAFEPE-furosemide (40mg) is not different when concentration versus time (C_{max} and t_{max}) is observed (Fig.3 and 4). Table 4 shows some variability among individuals. Nevertheless, it is consistent with what was reported by Straughn et al. (1986) and Wallwe et al. (1982). Following furosemide administration, when comparing UFPE'S and LAFEPE's data, the following blood plasma maximum
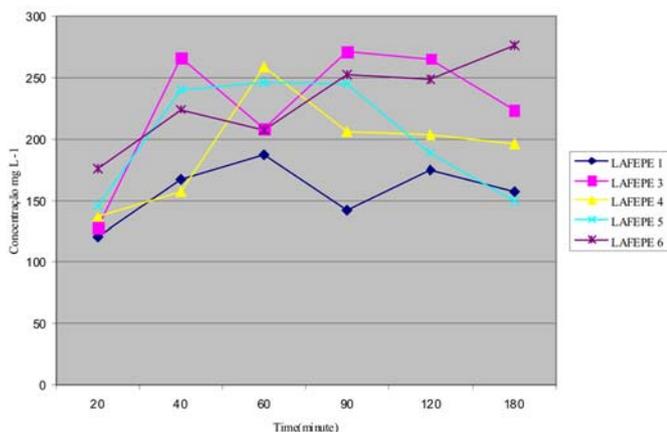
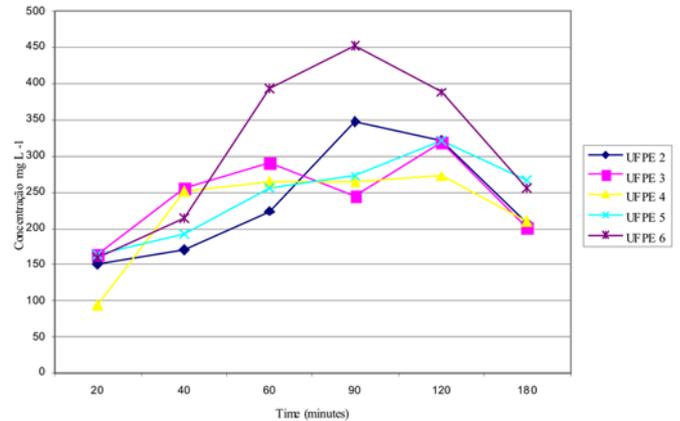Fig.3. Plasmatic liberation kinetics in street dogs for LAFEPE-furosemide (Furosemide concentration mg litre^{-1}).

Fig.4. Plasmatic liberation kinetics in street dogs for UFPE-tablets. (Furosemide concentration mg litre^{-1}).

concentration average values are reached: 288.390μg/mL for tablets furosemide (40mg) the UFPE product and 261.248μg/mL for the LAFEPE product. These values are not significantly different in relation to the 5% confidence limit. Regarding maximum concentration (T_{max}) time and global bioavaibility assessed by AUC means, there were no considerable differences as well. UFPE-furosemide displayed 743.492μg/mL.h as AUC average value whereas LAFEPE-furosemide had an average of 537.284μg/mL.h.

CONCLUSION

The liberation half times ($t_{1/2}$) for UFPE and LAFEPE furosemide vary between 60 and 90 minutes. The results obtained from this study indicate that following a single oral dose of two 40mg furosemide tablets, the differences shown by the data related to the test and reference products were considered statistically non-relevant within the 5% confidence level that, in turn, demonstrates that the two products are bioavailable.

The bioavailability data in plasma obtained after furosemide oral doses administration assure that it is possible to compare the bioequivalence of the products with absolute security.

Based on the comparison of *in vitro* data and on bioavailability blood plasma levels it is possible to guarantee the bioequivalence of the products.

Hence, there is an urgent need for the government to put into practice the current policy for the Brazilian generics which will make the bioequivalence studies a compulsory item by invigilating organs hence, improving security, efficiency and quality of commercialized veterinary products.

Finally, we believe that the bioequivalence study carried out through the product development (pharmaceutical development) compared to the reference product has proved to be a superior gain over two unknown products.

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